A Study to Investigate Factors Involved in the Development of Oesophageal Carcinoma

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A thesis submitted to the University of Glamorgan for the degree of Master of Philosophy.

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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ABSTRACT

Introduction: Dietary questionnaire studies have suggested that patients with oesophageal adenocarcinoma are deficient in antioxidants. It is unknown whether the same holds true for patients with the precursor lesion, Barrett's oesophagus. The current study considered the hypothesis that patients with Barrett's oesophagus were deficient in antioxidants compared to reflux patients without evidence of Barrett's oesophagus.

Bcl-2 is an inhibitor of apoptosis or programmed cell death and bax is a promoter. p53 is a protein regulating these. Studies have shown a bcl-2/bax ratio favouring bax expression to be predictive of a good response to neoadjuvant chemoradiotherapy; that expression of mutated p53 is predictive of a poor response.
The current study considered the hypothesis that the ratio of bcl-2/bax and the expression of p53 in oesophageal tumours could be used to predict the response to chemoradiotherapy.

Methods: Serum antioxidant profiles (copper, selenium, zinc, vitamins A, C & E, the carotenoids, and xanthophyll) were determined for patients with: Barrett's oesophagus (n=36), erosive oesophagitis (n=32) and patient controls (n=35).

The expression of bcl-2, bax and p53 in oesophageal tumour biopsies were ascertained by means of immunohistocytochemistry, and compared with the patients' known response to chemoradiotherapy.

Results: Patients with Barrett's oesophagus had significantly lower levels of selenium, vitamin C and β-cryptoxanthine compared to the other patient groups.

There was no difference demonstrated in the expression of bcl-2, bax, and p53 between two groups of patients, those responding well, and those responding poorly to chemoradiotherapy.

Conclusion: This study confirmed the hypothesis that patients with Barrett's oesophagus are deficient in certain antioxidants. It is speculated that antioxidant supplementation may play a role in the prevention of this condition and progression to neoplasia.

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ABBREVIATIONS

5- FU-	5- Fluorouracil
E.Pain-	Epigastric Pain
Gy-	Gray, a unit of radiation
H/B-	Heartburn
HDU-	High Dependency Unit
ITU-	Intensive Care Unit
NT-	Neoadjuvant Therapy
OGD-	Oesophagogastroduodenoscopy
Regurg.-	Regurgitation
RTX-	Radiotherapy
SCC-	Squamous Cell Cancer
TRG-	Tumour Regression Grade

GLOSSARY OF TERMS

Adenomata: A collection of neoplasms of glandular origin. Adenomas can grow from many organs including the colon, adrenal, pituitary, thyroid, etc. These neoplasms are benign, but some are known to have the potential, over time, to transform to malignancy (at which point they become known as adenocarcinoma).

Adjuvants: These are agents which modify the effect of other agents while having few if any direct effects when given by themselves. In this sense, they are very roughly analogous with chemical catalysts.

Cytology may refer to cytopathology, the cellular diagnostics of disease.

Endoscopy: This means looking inside and refers to looking inside the human body for medical reasons.

Gastroesophageal Reflux Disease (GORD): Defined as chronic symptoms or mucosal damage produced by the abnormal reflux of gastric contents into the oesophgus. This is commonly due to transient or permanent changes in the barrier between the oesophagus and the stomach. This can be due to incompetence of the lower oesophageal sphincter (LOS), also known as the cardiac sphincter, transient LOS relaxation, or association with a hiatal hernia.

Helicobacter pylori: A bacterium that infects the gastric mucosa. Many peptic ulcers and some types of gastritis are caused by H. pylori infection, although most humans who are infected will never develop symptoms. This bacterium lives in the human stomach exclusively and is the only known organism that can thrive in that highly acidic environment. It is helix-shaped (hence the name helicobacter) and can literally screw itself into the mucosa to colonize.

Histology: This is the study of tissue sectioned as a thin slice, using a microscope. It can be described as microscopic anatomy.

Leukopaenia: A low white cell count.

Ligand: In chemistry this is an atom or molecule that donates its electrons, or shares its electrons with one or more central atoms or ions.

Thrombocytopenia: The term for a reduced platelet count.

Metaplasia: The replacement of one differentiated cell type with another differentiated cell type.

Mitoses: The process of cells dividing. The number of mitoses per high power field of a pathological specimen correlates with the rate of cell division, and hence the degree of malignancy.

Mucositis: The swelling, and ulceration of the mucosal cells that line the digestive tract.

CHAPTER ONE

INTRODUCTION

1.1 THESIS OUTLINE

The present thesis consists of the following chapters.

Chapter One: Gives a general introduction to the risk factors involved in oesophageal cancer both in the initial development and subsequent progression.

Chapter Two: Outlines the published literature relevant to both studies.

Chapter Three: The first study described in this chapter was designed to determine whether antioxidant levels were lower in subjects with Barrett's oesophagus than in controls.

Chapter Four: This chapter outlines the second study which examined the expression of bcl-2, an apoptosis inhibitor, and p-53, an apoptosis regulator, in tumour specimens and correlated this with the response of that tumour to chemotherapy.

Chapter Five: This final chapter summarises the main findings from each study, discusses possible areas for further research and finally concludes.

1.2 INTRODUCTION

In recent years a worrying increasing trend in the incidence of oesophageal cancer has been noted. In some countries it has more than doubled in the last 20 years [1] . This however does not hold true for all types of oesophageal cancer, of which there are two main histological types. Squamous cell carcinoma usually occurs in the upper two thirds of the oesophagus, and is associated with smoking and alcohol, whereas adenocarcinoma of the oesophagus usually occurs in the lower third, and almost always arises in an area of Barrett's oesophagus. This is a condition caused by acid reflux from the stomach into the lower oesophagus. The normal lining of the oesophagus is damaged by the acid, and as it heals it undergoes a histological change, from the normal squamous epithelium into an abnormal columnar one. This is the same type as lines the stomach. It is the incidence of adenocarcinoma that has increased recently, the incidence of squamous cell cancer remaining the same. There is no clear evidence as to why there should have been such a dramatic increase, but it has been suggested it is due to the increase in obesity, and hence reflux disease [2] .

There have been many dietary studies investigating the relationship between diet, and both oesophageal squamous and adenocarcinoma. It has been shown beyond doubt that both histological types are more prevalent amongst people who consume a diet deficient in fresh fruit and vegetables [3-13]. There is also evidence to attest to the protective effects of antioxidants, with supplementation studies showing a decrease in the incidence in cancer amongst those patients receiving supplementation [14]. It has been shown that antioxidant deficiency is associated with an increased risk of progression of Barrett's mucosa to high-grade dysplasia, which is one step below adenocarcinoma, and patients with Barrett's oesophagus have been shown to have decreased Vitamin C levels both in the blood, and in the Barrett's tissue itself [15]. As

yet there is no published evidence looking at serum trace element levels (Selenium, Copper, Zinc) in patients with Barrett's oesophagus, or at the difference in antioxidant levels between patients with Barrett's, and those with oesophageal reflux, and concomitant mucosal damage.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 EPIDEMIOLOGY

The oesophagus is a muscular tube lined mostly by squamous mucosa. It extends from the pharynx to the cardia of the stomach, and in the adult is approximately 25cm long [16]. The lower 2 cm of the oesophagus is situated below the diaphragm, and is lined by columnar mucosa of gastric type. The junction between the two types of mucosa, the squamocolumnar junction is clearly visible on endoscopy, and is usually found at 40cm. This distance is measured from the incisor teeth [16].

There are two main histological types of carcinoma of the oesophagus. Squamous cell carcinoma, and adenocarcinoma [16]. Squamous cell tumours most commonly arise in the squamous epithelium in the upper two thirds of the oesophagus, and adenocarcinomas are most commonly found in the lower third. Adenocarcinoma arises from columnar mucosa. These tumours will have almost invariably developed on the basis of a Barrett's oesophagus [16]. Worldwide most carcinomas are squamous cell tumours, with adenocarcinomas accounting for 0.8-8.0% of all carcinomas, depending on the local epidemiology [17].

Cigarette smoking and alcohol consumption are important risk factors for squamous cell cancer in areas of the world that have a low to moderate risk [17]. A study undertaken by Vaughan *et al* in Seattle in 1995 investigated the use of alcohol and cigarettes by patients with both adenocarcinoma, and squamous cell carcinoma [18]. Both cigarette smoking, and alcohol consumption were found to be significant risk factors for both histological types, although the risk of developing squamous cell cancer amongst smokers is far higher than the risk of developing adenocarcinoma. Drinking over 21 UK units of alcohol per week was also a risk factor for developing squamous cell carcinoma [18].

In the year 2000 the World Health Organisation presented a detailed model to estimate cancer survival in different parts of the world. Oesophageal cancer was the tenth commonest cancer worldwide, with 386,612 new cases reported [19], and it accounted for 6% of all worldwide cancer deaths. It was preceded by lung, breast, stomach, colon, and liver, which were the first, second, third, fourth, and fifth commonest cancers that year [19]. In recent years the steadily increasing incidence of cancer of the lower oesophagus and upper stomach has been noted [1], in fact in some areas of the United States the incidence has doubled over the last 20 years, with increases in incidence in men ranging from 4% to 10 % per year. This rate of increase exceeded that seen for any other type of human cancer [1].

The incidence of squamous cell carcinoma has remained relatively stable with only minor increasing trends noted in Denmark and the Netherlands among men, with women demonstrating similar trends in Canada, Switzerland, and Scotland [20]. The situation in the United States is the same, with the incidence of squamous cell carcinomas remaining relatively constant over the last 15 years [18]. Contrary to this the incidence of adenocarcinoma has been rapidly increasing [1, 18]. Time-trends incidence of subsite-specific cancers were analysed by Vizcainio *et al* in order to examine the incidence patterns of the two major types of oesophageal cancer [20]. Their findings correlate with those above. There was an increase in the incidence of oesophageal adenocarcinomas in both sexes in the United States, Canada, South Australia, as well as in six European countries [20]. In the South Thames region of England there has been no significant change in the incidence rate of upper two-third carcinoma of the oesophagus, however lower third carcinoma has shown a marked increase for both sexes [21].

The rate of increase in incidence varies markedly according to region. An epidemiological study using information from the U.S. Surveillance Epidemiology, and End Results (SEER) cancer registry from the years 1973-1998 has found the rate of increased incidence of adenocarcinoma in Seattle to be over twice that of Utah, the rates being 800%, and 300% respectively [22]. The rate of increase in incidence of adenocarcinoma in Europe (Germany) ranges from 248% in Brandenburg to 432% in the Saarland [23]. Although both these papers quoted have not postulated as to why there should be such a geographical difference in the observed increase in incidence, it has been proposed that variations in the coding, classification and detection of gastro-oesophageal malignancy may have contributed partially to the observed trends [2]. It is also entirely possible for there to be geographical variation in the factors promoting the development of oesophageal carcinoma, namely alcohol, smoking, obesity, and diet.

2.2 REASONS FOR INCREASING INCIDENCE

Barrett's oesophagus is a pre-malignant condition, which can give rise to adenocarcinoma, and gastro-oesophageal reflux disease is a precursor to Barrett's [16]. Factors promoting the development of both have been proposed to explain the rising trends discussed above; these include the declining rates of Helicobacter pylori infection, obesity and dietary factors [2]. In a Swedish nationwide case control study, gastro-oesophageal reflux and obesity were identified as strong and independent risk factors for oesophageal adenocarcinoma [24]. With increasing duration and severity of reflux symptoms and with increasing body mass index the risk increased in a dose dependent manner. When combined, reflux symptoms and obesity entailed greatly increased risk estimates. The author proposes possible reasons for the increasing

incidence of adenocarcinoma of the oesophagus, and these include an increase in the prevalence of reflux disease, and the increasing prevalence of obesity [24]. A Swedish epidemiologic investigation by the same author of the possible association between gastro-oesophageal reflux and adenocarcinoma of the oesophagus has corroborated these findings [25]. Among persons with recurrent symptoms of reflux, as compared with persons without such symptoms, the odds ratios were 7.7 for oesophageal adenocarcinoma. The more frequent, more severe, and longer lasting the symptoms of reflux the greater the risk. It was found that amongst persons with long-standing and severe symptoms the odds ratios were 43.5 for oesophageal adenocarcinoma [25].

2.3 BARRETT'S OESOPHAGUS

The major risk factor for the development of adenocarcinoma at the lower oesophagus and gastroesophogeal junction is the condition known as Barrett's oesophagus [26]. This was named after a British surgeon Norman Rupert Barrett, who worked at St Thomas's Hospital, London from where he had graduated in 1928. It is postulated that gastric juice refluxes into the lower oesophagus and this can injure the oesophageal squamous epithelium. When this injury heals through a metaplastic process an abnormal columnar epithelium (the same type as lines the stomach) replaces the injured squamous epithelium and this results in the condition of Barrett's oesophagus [26].

Damaged lower oesophageal epithelium was first recognised in 1906 by a pathologist called Tileston [27], who reported on several patients with peptic ulcer of the oesophagus, and the close resemblance of the mucous membrane surrounding the ulcer to that normally found in the stomach. It was firstly thought that this was due to

the proximal part of the stomach being tethered in the chest by a congenitally short oesophagus and this was supported by Barrett in 1950 [28]. By 1953 it was argued that the columnar-lined intrathoracic structure was in fact oesophagus, due to the lack of a peritoneal covering and the presence of islands of squamous epithelium and submucosal glands [29]. Barrett suggested the condition be called 'lower oesophagus lined by columnar epithelium' [30]. It has since been known as Barrett's oesophagus. This pre-malignant condition ie Barrett's oesophagus can be recognised at the time of upper gastrointestinal endoscopy and confirmed by biopsies of the oesophagus. It is found in approximately 12% to 18% of patients undergoing upper endoscopy for symptoms of reflux [31, 32]. Modern data indicate that patients with Barrett's oesophagus develop oesophageal adenocarcinoma at the rate of 0.5% per year, a rate that is more than 30-fold higher than that of the general population [33].

2.4 PROGNOSIS

The prognosis of any patient diagnosed with oesophageal cancer is dismal. In one report from Europe (Germany) the five-year survival of all patients with oesophageal cancer was less than 10% for the period 1971-1995 [23]. In Northern America the median survival is 14.5 months, for those patients receiving maximum treatment in the form of surgery and chemoradiotherapy [34]. In a survey of oesophageal and all gastric cancers in Wales only 25% of all patients were alive at two years [35]. In a report by the World Health Organisation for the year 2000 the global incidence of oesophageal cancer was 386, 612, with a mortality of 350, 841 [19]. This means that for any given year almost the same number of people die from oesophageal cancer as are diagnosed, outlining the poor prognosis.

The only opportunity of a cure for oesophagastric cancer is surgical resection. This is a major procedure which carries a mortality rate of around 8% [36]. In a review of 126 patients having a resection for oesophageal carcinoma Mariette et al achieved an overall three and five year survival rate of 41% and 25% respectively [37]. Thus surgical removal of the tumour offers a much improved overall survival rate, but only a very small proportion of patients are eligible for resection. In the Welsh survey 33% of patients were able to be treated this way [35], the remainder being excluded on the basis of poor physiological reserve, liver metastases and local invasion. The majority of patients are inoperable at the time of presentation.

2.5 FACTORS WORSENING PROGNOSIS

There are factors which worsen prognosis in oesophageal cancer. The presence of lymph node metastases significantly worsens the patients' prognosis [38]. A Japanese study in 2000 assessed the survival of patients having had an oesophageal resection and who were found to be lymph node free of metastases at the time of operation. The 1, 3, 5, and 10 year survival rates were 86%, 73%, 67%, and 35% respectively [39]. A further Japanese study looked at the prognosis of patients with zero, one to three, four to seven, and eight or more lymph nodes involved. The 5-year survival rates were 53%, 34%, 17%, and 0% respectively [40]. It is obvious that patients with fewer metastases have better long term survival, but unfortunately many patients present at an advanced stage hence the poor prognosis.

2.6 NEOADJUVANT THERAPY (NT)

Chemoradiotherapy can be used in the neoadjuvant or palliative setting for oesophageal cancer and also theoretically to treat micrometastases (microscopic metastases that are not detectable with standard imaging). Currently neoadjuvant chemoradiotherapy is given in an attempt to downsize advanced tumours in order that surgical resection may be more successful. The drugs most usually used for neoadjuvant therapy are cisplatin plus 5-fluorouracil. These work by inhibiting the mitoses of tumour cells. Tumour cells divide much more frequently than normal cells in the body and this is why the drugs target them. Unfortunately the drugs also have an effect on normal cells in the body, particularly those that divide frequently, such as gastrointestinal mucosa, and bone marrow. This accounts for unpleasant side effects. These include nausea and vomiting, diarrhoea, leukopaenia, thrombocytopaenia, electrolyte imbalance, febrile neutropaenia and mucositis, even death has been documented [41]. The drugs are also nephrotoxic. [42]. Furthermore there are also complications related to the long-term infusion lines used for the administration of the drugs. Most commonly sepsis and line infection occur [43].

Radiotherapy to the oesophageal tumour is given in fractions of 45 Gy, administered in 25 fractions over a five-week period, concurrently with the third and fourth cycles of chemotherapy [44]. Radiation portals with a five-six cm margin above and below the tumour are recommended [45]. This field will inevitably include the heart and lungs. Radiotherapy can damage normal tissues, and computed tomography frequently demonstrates radiation-related lung damage adjacent to the mediastinum [45]. Radiotherapy to the lungs can result in increasing impairment of gas exchange [46], and cardiac complications are a particular problem with radiation treatments to the mediastinum and breast [47].

Neoadjuvant therapy (NT) is a difficult area to review. Preoperative chemoradiotherapy has been used in an attempt to decrease tumour activity, increase resectability rates and improve disease-free and overall survival [48]. The only real measure of its worth is post-op survival and this appears to be greater in patients who have had a complete histological response to the therapy, namely in whom there are no viable tumour cells in the resected specimen. The percentage of patients having a complete histological regression to chemoradiation varies, with figures of 15%, [49] to 33%, [50] quoted in the literature.

2.7 MANDARD (TUMOUR REGRESSION GRADE)

Mandard *et al* 1994 carried out a pilot study to determine if pathologic assessment of tumour regression correlated with disease-free survival [51]. Semi-serial sections of resected specimens from patients treated with pre-operative cisplatin and radiotherapy, who then underwent surgery were examined. All specimens were allocated a tumour regression grade (TRG). This was quantified as follows: TRG 1 (complete remission) showed absence of residual cancer with fibrosis extending through the different layers of the oesophageal wall. TRG 2 was characterised by the presence of rare residual cancer cells scattered through the fibrosis. TRG 3 was characterised by an increase in the number of residual cancer cells, but fibrosis still predominated. TRG 4 showed residual cancer outgrowing fibrosis, and TRG 5 was characterised by absence of regressive changes. 93 consecutively resected specimens were examined. Of these 42% were TGR 1-2, 20% were TGR 3, and 33% were TGR 4-5. After multivariate analysis, only tumour regression (ie TRG 1-3 vs TRG 4-5) remained a significant ($p<0.001$) predictor of disease free survival.

Conversely a second paper, the research for which was carried out in Ireland, examined the TRG in similar patients and found that TRG had no significant effect on survival (p=0.06) [52].

2.8 RESPONSE TO NEOADJUVANT THERAPY

In a recently published (2002) Japanese non-randomised study of patients with advanced (T4) oesophageal squamous cell cancer it was found that after neoadjuvant therapy (NT) and successful surgical resection, the five-year survival rate was prolonged significantly in patients particularly those who achieved a complete response (71% compared to 32% for a partial response) The remaining 7% of patients had no response and the five-year survival rate was 0% [53]. This finding has been supported by a European randomised controlled trial, again of patients with oesophageal squamous cell cancer. Patients responding to chemoradiotherapy and having a surgical resection had significantly better 3 and 5-year survival rates (74% and 60%), as compared to non-responders to chemoradiotherapy, who also had a surgical resection. (24%, and 12% respectively; p=0.0002) [54]. Walsh *et al* (1996) conducted a prospective, randomised trial comparing surgery alone with combined chemotherapy, radiotherapy and surgery. Fifty-five patients with oesophageal adenocarcinoma had multimodal therapy, and fifty-five patients, again with adenocarcinoma had surgery alone. 42% of patients treated with multimodal therapy had positive nodes or metastases as compared with 82% undergoing surgery alone ($p<0.001$). The median survival of patients assigned to multimodal therapy was 16 months, as compared with 11 months for those assigned to surgery (p=0.01). At one, two, and three years, 52, 37, and 32 percent, respectively of patients assigned to multimodal therapy were alive, as compared with 44, 26, and 6 percent of those

assigned to surgery. The authors concluded that multimodal treatment was superior to surgery alone for patients with resectable adenocarcinoma of the oesophagus [55].

The Medical Research Council Oesophageal Cancer Working Group conducted a randomised controlled trial comparing surgical resection with or without preoperative chemotherapy in oesophageal cancer of any cell type. Clinicians could choose to give radiotherapy irrespective of randomisation. This was a large trial with 802 patients recruited. There were 400 in the chemotherapy and surgery group (CS) and 402 in the surgery alone group (S). Median survival was 512 days in the CS group compared with 405 days in the S group. Two-year survival rates were 43% in the CS group and 34% in the S group. Overall survival was better in the CS group ($p=0.004$). The investigators concluded that two cycles of preoperative cisplatin and fluorouracil improve survival without additional serious adverse events in the treatment of respectable oesophageal cancer [56].

Urschel [57] conducted a meta-analysis of randomised controlled trials that compared neoadjuvant chemoradiation and surgery to surgery alone for resectable oesophageal cancer. Nine trials, including 1116 patients were selected. A complete pathological response to chemoradiation occurred in 21% of patients. The three-year survival benefit was most pronounced when chemotherapy and radiotherapy were given concurrently, instead of sequentially. Chemoradiation was also associated with a lower rate of oesophageal resection, but a higher rate of complete resection [57].

It is possible preoperatively to determine patients who are likely to have achieved a pathological response by measuring the reduction in tumour size with endoscopic ultrasound (EUS), post NT. Chak [34] for example reported that patients classed as responders on repeat EUS had a median survival of 17.6 months, as compared to 14.5 months for non-responders; $p=<0.005$. Survival was significantly

longer in responders compared with non-responders in the patient subgroup who underwent surgical resection (19.7 months vs. 14.6 months; p=<0.005) [58].

The other area in which NT seems to be effective is in the treatment of locally advanced tumours. The survival has been found to be prolonged in squamous cell carcinoma node-positive patients with a median survival of 12 months compared to 19 months, for those patients having resection alone, or resection + NT; p=0.0193 [50] and it has also been found to improve survival in T4 tumours, with NT patients having a better 5-year survival [59].

2.9 CONCERNS REGARDING NEOADJUVANT THERAPY

There are concerns as to the use of NT. Firstly, is the issue regarding the use of the patients' remaining survival time. Some would advocate that the patient would be best served by having a comfortable remaining survival time rather than have it taken up by NT, with all its attendant side effects, and repeated hospital visits.

Secondly, there have been studies, finding there to be no benefit whatsoever to the patient with regards to improving their survival time [48, 58, 60, 61]. Using the Cochrane database reviews of 14 randomised controlled trials and one meta-analysis of pre-operative chemotherapy versus surgery alone Malthaner concluded that there is no strong evidence to recommend pre-operative chemoradiotherapy in the treatment of surgically resectable carcinomas of the thoracic oesophagus [48].

A further study carried out in Hong Kong of 83 consecutive squamous cell oesophageal cancer patients having NT, followed by surgical resection concluded that there was no added benefit in the overall survival of these patients [58]. This conclusion has been supported by a paper produced by the Sylvester Comprehensive Cancer Center, Miami, Florida [60]. In this study 72 patients underwent oesophageal

resection. Patients were divided into three groups according to the type of preoperative treatment; group 1(n=44) surgery alone; group 2(n=18) neoadjuvant 5- fluorouracil chemotherapy; group 3(n=9) neoadjuvant 5- fluorouracil based chemotherapy, and external beam radiation. One patient received radiotherapy alone. It was found that neoadjuvant therapy failed to improve survival rates and preoperative chemoradiation was associated with a high perioperative mortality rate. Finally, a randomised trial conducted in New York assessed the overall survival of patients randomised to chemoradiotherapy and surgery or surgery alone. It was found that preoperative chemotherapy with a combination of cisplatin and 5- fluorouracil did not improve the overall survival among patients with adenocarcinoma of the oesophagus [61].

Lastly, there are concerns as to whether the giving of NT may actually cause an increased morbidity and mortality in the post-operative period. Unfortunately it may be that if the patients' tumour cells are sensitive to chemotherapy and radiotherapy then their normal, healthy tissues may also be sensitive. In other words, if a patient responds to treatment they may be at a higher risk of post-operative complications. It has been shown that patients who received neoadjuvant therapy had a higher rate of pneumonia [59], and they may be susceptible to intractable infections [62]. It has also been found to reduce the physical performance of the patient, which can be taken as a predictor of the subsequent postoperative risk [63]. However, there are other studies, which have found there to be no difference in complication rate [64, 65]. The previously discussed Medical Research Council Oesophageal Cancer Working Group study found there to be no difference in the post-operative complications reported in the surgery and chemotherapy group and the surgery alone

group. Finally NT is expensive and it may be that this is a waste of resource if there is no real advantage to the patient by its application.

2.10 bcl-2, bax AND p53

It would obviously be an advantage if it were possible to predict the patients who will respond to chemoradiotherapy, as currently only approximately one third of patients have an absence of cancer cells histologically. This conversely means that two thirds of patients will not have a histological response, having residual cancer cells, and could be spared toxic drug regimes unlikely to confer any survival advantage. The response to chemoradiotherapy depends on how readily tumour cells can be triggered to apoptose.

Apoptosis is defined as an active, physiologic cascade of events, characterised by cell shrinkage due to dehydration, nuclear fragmentation, and membrane blebbing [66]. Cell death marked by cellular swelling should be called oncosis, whereas the term necrosis refers simply to cell death [67]. Apoptosis and oncosis are therefore pre-mortal processes [67]. The first microscopic denominations for cell death appeared in 1879 with the introduction of the terms karyorhexis indicating the disintegration of the nucleus, and karyolysis describing the disappearance of the nucleus. In 1885 the term chromatolysis was coined to describe the disappearance of the nucleus [67].

Apoptosis is a form of intentional suicide based on a genetic mechanism. It is characterised by morphological as well as biochemical criteria [68]. Morphologically the cell shrinks, and becomes denser. The chromatin becomes packed into smooth masses applied against the nuclear membrane, creating curved profiles that have inspired terms such as half-moon, horse-shoe, sickle, and ship-like. The nucleus may also break up, and the cell emits processes that often contain nuclear fragments. These

processes tend to break off and become apoptotic bodies, which may become phagocytosed by macrophages or neighbouring cells or remain free. The cell may also shrink into a dense rounded mass, as a single apoptotic body [68]. There is little or no swelling of mitochondria or other organelles. The DNA is biochemically broken down, due to specific cleavage. The process is under genetic control, and can be initiated by an internal clock, or by extracellular agents such as hormones, cytokines, killer cells, and a variety of chemical, physical, and viral agents [68].

There is a misunderstanding between the terms apoptosis, and programmed cell death. Two different scenarios are involved in apoptosis. Firstly a programme to carry out suicide, and secondly a programme to trigger suicide. The phenomenon properly known as programmed cell death refers to situations in which cells are programmed to die at a fixed time. Such is the death on schedule of certain clusters of cells in the embryo. For example in the chick embryonic plate, a group of cells has to die at a precise time to help create the outline of a wing. These cells die on schedule even if they are transplanted elsewhere in the embryo [68].

Ischaemic cell death is characterised by swelling; thus it is defined by a name that refers to swelling, ie oncosis [68]. This is a form of cell death accompanied by cellular swelling, organelle swelling, blebbing, and increased membrane permeability. Its mechanism is based on failure of the ionic pumps of the plasma membrane. It is caused typically by ischaemia and possibly by toxic agents that interfere with ATP generation or increase the permeability of the plasma membrane. It is usually accompanied by karyolysis [68].

Three mechanisms are known to be involved in the apoptotic process; a receptor-ligand mediated mechanism, a mitochondrial pathway, and a mechanism in which the endoplasmic reticulum plays a central role [67]. All three mechanisms

activate caspases which are responsible for the characteristic morphological changes observed during apoptosis. Apoptosis can be induced by binding of a ligand on a specific receptor which is located on the cell membrane. Procaspases are then converted into active caspases after the receptor is activated by ligand binding [67]. These specific receptors contain a cytoplasmic domain responsible for the signal transduction in apoptosis. This domain is called the death domain.

The receptor-ligand mediated apoptotic pathway is not the only mechanism in the apoptotic process. Certain cytotoxic agents, such as nitrogen monoxide, and radiation, cause apoptosis in another manner involving the mitochondria, and more specifically the mitochondrial protein cytochrome c. [67] This is located on the outside of the inner mitochondrial membrane, and in the intermembrane space. It has an important function in the intracellular electron transport chain reaction for the production of ATP. During apoptosis cytochrome c is released in the cytosol. There it binds to the apoptosis protease activating factor. These form a complex together, activate procaptases and captases leading to the known morphological consequences [67].

The release of cytochrome c into the cytosol is regulated by proteins coded by genes of the bcl-2 (B-cell lymphoma) family. Bcl-2, and bax are members of this family. Bcl-2 is an apoptosis inhibitor which is found in vertebrates, and it is thought to inhibit the working of cytochrome c rather than its release [67]. Bax is an apoptosis promoter, and does this by releasing cytochmome c, although it is still unclear how it achieves this. It is possible that bax and bcl-2 regulate mitochondrial permeability via a megachannel which is also called mitochondrial permeability transition pore. This channel is built up by proteins of the inner and outer mitochondrial membrane and by intermembrane proteins. The opening of this channel results in an influx of ions with

subsequent swelling of the mitochondrion. This causes breaks in the outer membrane while the inner membrane remains intact because it has a larger surface. Cytochrome c escapes through the outer membrane breaks and enters in the cytosol [67]. The formation of a megachannel also occurs in oncosis/necrosis, but in these latter processes the cellular metabolism is already damaged to the extent that activation of captases is no longer possible and the cell has no control of its own death mechanism [67].

The p53 tumour suppressor gene codes for the p53 protein. This gene is mainly expressed when DNA damage occurs by radiation or chemotherapeutics [67]. The subsequently produced p53 protein, if present in high amounts, increases the number of bax transcripts, and subsequently induces apoptosis [67]. Wild-type p53 protein also mediates apoptosis by directly activating the expression of bax and by indirectly inhibiting the expression of bcl-2 [69, 70].

Apoptosis is an energy dependent process, whereas oncotic cell death is independent of captase activation, and occurs subsequent to ATP depletion [71]. Intracellular ATP plays an important role in the execution of apoptosis and not of oncosis. Furthermore it has been shown that necrosis appears exclusively in the presence of total glucose deprivation accompanied by total loss of ATP. Thus it appears that oncosis occurs at low to zero ATP levels, and apoptosis can only occur in the presence of higher intracellular energy stores [71].

Katada *et al*, [72] found overexpression of bcl-2 in 72% of Barrett's metaplastic lesions and in 100% of Barrett's with low-grade dysplasia. Rioux-Leclerq *et al* [73] also reported loss of bcl-2 expression in Barrett's metaplasia with high grade dysplasia. These results suggest two possible explanations. Firstly that inhibition of apoptosis by overexpression of bcl-2 occurs early in the dysplasia-

carcinoma sequence and secondly that dysplastic and cancerous cells are capable of evading apoptosis through cellular mechanisms other than those involving bcl-2 inhibition of apoptosis [72].

There have been studies looking at the expression of p53 in oesophageal tumour specimens. It been found to harbour mutations in more than 50% of both squamous cell and adenocarcinomas of the oesophagus [74]. Further studies have been undertaken to see whether expression of p53 can be used to predict the response to chemoradiotherapy. The results of these are somewhat contradictory, with one American study concluding that overexpression of p53 is associated with decreased responsiveness to induction chemoradiotherapy in patients with oesophageal adenocarcinoma, but that no such association exists in patients with oesophogeal squamous cell carcinoma [75].

A Japanese study however, assessed the presence of p53 mutation in 40 patients having chemoradiotherapy for oesophgageal carcinoma and found that 24 patients had a p53 mutation. 13 patients achieved a complete response to the chemoradiotherapy. The survival rate in the 24 patients with p53 mutation did not differ significantly from that in the 16 patients without the mutation [76].

A further Japanese paper followed a different approach. Human oesophageal tumour cells bearing mutated p53 were retrovirally transduced with wild-type p53. The transduced cells, which stably expressed wild-type p53 proliferated at the same rate as parental cells. However the sensitivity to radiation was significantly improved and the tumour cells became markedly susceptible to chemotherapy compared to the parental cells. Therefore forced expression of wild-type p53 gene can increase the sensitivity to DNA damage in oesophageal cells [77].

Loss of bax expression has been reported to be associated with poor response to chemoradiotherapy in breast cancer [78]. The situation regarding bcl-2 and bax as regards oesophageal cancer is not as clear-cut. There have been several studies, all assessing the effect of the two proteins individually. The methodology of these was the same. The original biopsy specimens of the tumours, or samples of the surgically resected specimen were used for immunohistochemical detection of bcl-2 and bax and these were correlated with the known response to chemoradiotherapy. Ikeguchi *et al* used immunohistochemical detection of bax in surgical specimens from the primary oesophageal tumours [79]. Bax expression was determined by two independent pathologists who had no knowledge of the patients. The percentage of positive tumour cells was determined by assessing the whole tumour section. Lymphocytes and normal oesophageal epithelium within the sample showed strong bax staining intensity and this served as an internal control for bax staining. The specimen was decided as bax positive when more than 75% of cancer cells showed unequivocal and strong immunoreaction. Bax expression correlated with favourable prognosis in 57 patients with postoperative chemoradiotherapy [79]

Raouf *et al* in 2001studied oesophageal tumour resection specimens [80]. They were stained for p53 and bcl-2. Sections of human tonsil and colon adenocarcinoma known to be positive for bcl-2 and p53 were used as positive controls. Out of 2000 tumour cells the number showing moderate to intense immunostaining was counted, and the percentage positivity recorded. They found bcl-2 (apoptosis inhibitor) expression was not associated with tumour response or resistance to chemoradiotherapy [80]. In a further study Sarbia *et al* stained presurgical resection tumour biopsies for p-53, bcl-2, and bax. Using light microscopy the immunohistochemical expression was examined by one observer without

knowledge of the clinical outcome. The percentage of positive tumour cells was determined by assessing the whole tumour section. Each sample was assigned to one of the following categories. 0 (0-4%), 1 (5-24%), 2 (25-49%), 3 (50-74%), and 4 (75-100%). Additionally the intensity of immunostaining was determined as negative, weak, and strong. They found no correlation between the expression of bcl-2, bax and the response to chemotherapy [81].

The ratio of bcl-2 to bax appears to be more promising in predicting the effectiveness of radiotherapy. In a study of prostate cancer immunohistochemical analysis of prostate sections for bcl-2 and bax was carried out [82]. Cell counts were performed manually using a 10 x 10 microscope grid at x 400 magnification. Both the number of immunoreactive cells and the total number of cells were determined by visual inspection of four different fields per section. The percent immunoreactivity for bcl-2 and bax proteins was based on the ratio of positive cells to the total number of cells counted. All counting was performed by two different individuals unaware of patient outcome. An elevated bcl-2/bax ratio predicted an increased risk of the cancer failing to respond to radiotherapy and indicated that the ratio could be used clinically to predict the radioresistance of prostatic tumours [82]. In a study of oesophageal cancers and radiotherapy, again using biopsy specimens, the specimens were considered immunopositive when >10% of tumour cells had definite immunostaining [83]. Ratios of bcl-2 to bax were determined as a quotient of respective immunopositive areas. In their study the group responding to radiotherapy had a bcl-2/bax ratio favouring bax expression, although the actual values of bcl-2 and bax showed no relationship to effectiveness of radiotherapy.

2.11 SCREENING

An area of dilemma is that of screening for oesophageal cancer. This is because there is no consensus as to the best treatment when the diagnosis is made. It would seem logical that the only way of improving the survival from oesophageal cancer is to detect it early. The Japanese have some of the best survival results in the world and they advocate a screening programme of endoscopic examination using iodine staining for high-risk groups, such as alcoholics [84], this is obviously not practised in the UK, as the incidence of oesophageal cancer here is far lower than that in Japan and such a screening programme would not be cost effective. There are also ethical considerations in such a programme.

One way of improving the pick-up rate for screening is to target high-risk groups such as those with Barrett's oesophagus. It is hoped that this will identify cancers at an earlier stage, at which time they may be potentially curable. Indirect evidence that surveillance is beneficial comes from observational studies, showing that cancers discovered during surveillance are less advanced and associated with longer survivals compared those detected during endoscopies performed for evaluating cancer symptoms [33].

Even though Barrett's is a strong risk factor for oesophageal adenocarcinoma, research has found that survival for patients with Barrett's does not differ significantly from that for matched individuals in the general population. For example Eckardt *et al* [85] compared a group of 60 patients with Barrett's oesophagus with 2 other groups of patients, the first group was 60 patients with achalasia, and the second was 60 patients with Schatzki's ring. Achalasia is a condition in which dilatation of the oesophagus occurs with retention of solids and liquids [17]. Schatski ring is a narrowing of the lower end of the oesophagus due to a ridge of mucosa or a fibrous

membrane. This may be asymptomatic but can occasionally produce dysphagia [86]. The main finding of the study was that overall survival did not significantly differ between the three groups. Additionally survival in the patient groups was not significantly different from that of the general population. There were 11 deaths in the Barrett's group and although 2 subjects in this group developed adenocarcinoma, none of the deaths was attributed to cancer.

This may be explained by the low absolute rather than the high relative incidence of cancer in Barrett's oesophagus [33]. Studies of survival in patients with Barrett's oesophagus have been undertaken predominantly in older men, for whom the risk of death from common lethal disorders such as myocardial infarction and stroke far exceeds their annual risk of death due to oesophageal carcinoma [33].

In another recent (2001) study of 335 patients with Barrett's 75 died of unrelated causes 47 had other diseases limiting survival and 59 were over 75. All women, and men with Barrett's <3cm in length were excluded, thus only 52 of the original 335 patients (less than 20%) would have benefited from surveillance [87]. This has been supported by a further study, in which 59 patients with Barrett's were followed. Only two developed oesophageal adenocarcinoma, representing only one case in 209 patient years and over 90% of the patients died as a result of unrelated causes [88]. In a recent cohort study of Barrett's oesophagus patients among 589 adenocarcinoma patients only 3.9% had a Barrett's oesophagus diagnosed before their cancer [89]. Thus, even if current surveillance techniques are effective, they are unlikely to substantially inpact the population's mortality from oesophageal cancer.

There is however some evidence available to suggest that endoscopic screening of Barrett's oesophagus is cost effective. Streitz *et al* [90] undertook a cost analysis of endoscopic surveillance to detect adenocarcinoma in Barrett's oesophagus

compared with mammography used to detect occult carcinoma of the breast. The cost of detecting a case of adenocarcinoma in Barrett's oesophagus was $37,928 and of detecting a case of breast cancer was $54,513. Endoscopic surveillance of patients with Barrett's oesophagus compared favourably with surveillance mammography and should be considered to be as cost effective.

Evidence is also available to show that screening of patients with Barrett's oesophagus should extend life. Provencale *et al* [91] recommend five yearly endoscopic screening. This cost $98,000 per quality adjusted life year (QALY) gained, as compared with heart transplantation ($160,000 per QALY gained) and screening for tuberculosis ($216,000 per QALY gained). No matter what the evidence is for or against, some practitioners prefer to perform surveillance rather than miss curable neoplasms [33].

Surveillance-detected Barrett's oesophagus associated adenocarcinomas are accompanied by low-stage disease and improved survival according to the findings published by Corley *et al* [89]. This was a cohort study of 23 Barrett's oesophagus patients, among 589 oesophageal or gastric cardia adenocarcinoma patients diagnosed between 1990-1998. Among these 23 patients 73% of the surveillance-detected cancer patients were alive at the end of follow up, compared with none of the patients without surveillance-detected cancers ($p=0.001$). All surveillance-detected cancer patients had low-stage disease and none died directly from cancer.

These findings are supported by van Sandick *et al* [92]. A clinicopathological comparison was made between patients who initially presented with oesophageal adenocarcinoma ($n=54$) and in those in whom the cancer had been detected during surveillance of Barrett's oesophagus ($n=16$). Surgical pathology showed that surveyed patients had significantly earlier stages than non-surveyed patients ($p=0.0001$). Only

one surveyed patient (6%) versus 34 non-surveyed patients (63%) had nodal involvement (p=0.0001). Two-year survival was 85.9% for surveyed patients and 43.3% for non surveyed patients (p=0.0029).

2.12 ANTIOXIDANTS

Reactive oxygen species (oxidants) are products of partial reduction of molecular oxygen. They are formed in the body as a result of regular metabolic transformations. An important source of reactive oxygen species is transformations associated with cytochrome p 450. This cytochrome metabolises a number of various substances, including drugs, and aromatic hydrocarbons present in smog and tobacco smoke. Another important source of reactive oxygen species is the oxidative stress resulting from inflammatory processes, which is associated with the metabolism of every organism [93].

Malignancies fall within the category of diseases whose pathogenesis is postulated to be related to the reactive oxygen species effects. Their intensified production, deficiency of antioxidants or impairment of the repair mechanism result in the increase in processes responsible for the development of malignancies [94].

To counter the effect of oxidising agents, collectively referred to as oxidants, cells have antioxidant systems. These include enzymes that break down oxidants and molecules that react with free radicals. Antioxidants are naturally occurring trace elements, collectively referred to as micronutrients. These include substances as varied as zinc, selenium and copper. Vitamins such as vitamins A, C and E are also antioxidants. These can all be found in various food substances in the diet.

Micronutrient deficiency can mimic radiation (or chemicals) in damaging DNA by causing single and double strand breaks or both. Double strand breaks are a

strong predictive factor for human cancer. Those micronutrients whose deficiency mimics radiation include Vitamin C, E and Zinc [94].

2.13 SELENIUM

Selenium is a trace element that is essential in small amounts, but can be toxic in larger amounts [95]. The main dietary sources of selenium are brazil nuts and grains. It is also found in fish, meat and bread. The recommended dietary allowance of selenium is 55mcg a day [96]. Humans and animals require selenium for the function of a number of selenium-dependent enzymes, also known as *selenoproteins* [97].

Thioredoxin reductase is such a selenoprotein. In conjunction with the compound thioredoxin, thioredoxin reductase participates in the regeneration of several antioxidant systems, possibly including vitamin C [98]. Selenium is also a component of gluathione peroxidase. Four selenium-containing glutathione peroxidases (GPx) have been identified: cellular or classical GPx, plasma or extracellular GPx, phospholipid hydroperoxide GPx and gastrointestinal GPx. Although each GPx is a distinct selenoprotein, they are all antioxidant enzymes that reduce potentially damaging reactive oxygen species, such as hydrogen peroxide and lipid hydroperoxides, to harmless products like water and alcohols by coupling their reduction with the oxidation of glutathione [99].See figure one page 30.

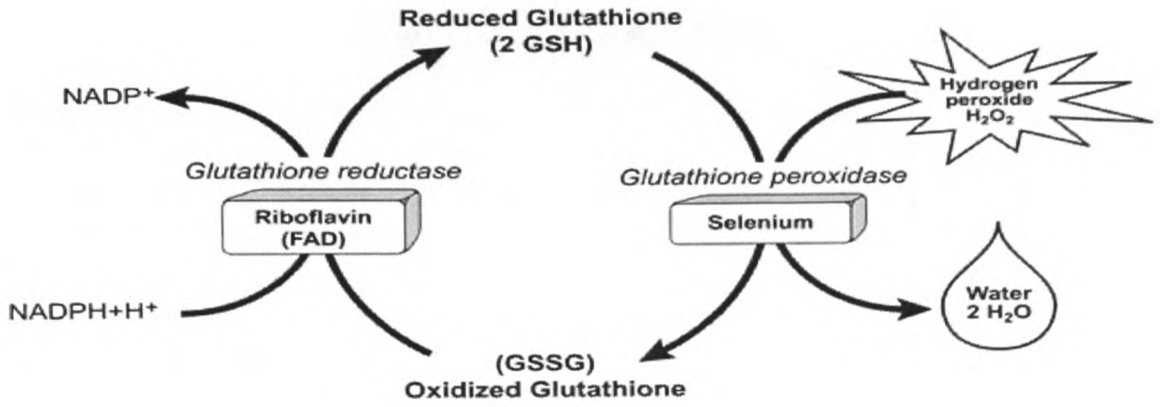

Figure One. Function of Selenium and Glutathione Peroxidase in the reduction of free radicals. [100]

Thyroid hormone deiodinases are selenium dependent. The thyroid gland releases very small amounts of biologically active thyroid hormone (triiodothyronine or T_3) and larger amounts of an inactive form of thyroid hormone (thyroxine or T_4) into the circulation. The conversion of the inactive form to the active form is catalysed by thyroid hormone deiodinases. This makes selenium an essential element for normal development, growth and metabolism through the regulation of thyroid hormones [99].

As an integral part of the glutathione peroxidases and thioredoxin reductase, selenium probably interacts with every nutrient that affects the prooxidant/antioxidant balance of the cell. Other minerals that are critical components of antioxidant enzymes include copper and zinc. Selenium as gluthathione peroxidase also appears to support the activity of vitamin E in limiting the oxidation of lipids [95].

Insufficient selenium intake results in decreased activity of the glutathione peroxidases. Even when severe, isolated selenium deficiency does not usually result

in an obvious clinical illness. However, selenium deficient individuals appear to be more susceptible to additional physiological stresses [95]. Clinical selenium deficiency has been identified in chronically ill patients who were receiving total parenteral nutrition (TPN) without added selenium for prolonged periods of time. Muscular weakness, muscle wasting and cardiomyopathy (inflammation and damage to the heart muscle) have been observed in these patients. TPN solutions are now supplemented with selenium to prevent such problems [95]. People who have had a large portion of the small intestine surgically removed or those with severe gastrointestinal problems, such as Crohn's disease, are also at risk for selenium deficiency due to impaired absorption [95].

2.14 ZINC

Zinc is an essential trace element for all forms of life. The main dietary sources of zinc are shellfish, beef and other red meats. It is also found in nuts and legumes (peas and beans). The recommended dietary allowance of zinc is 11 mg/day [100]. Numerous aspects of cellular metabolism are zinc-dependent. Zinc therefore plays important roles in growth and development, the immune response, neurological function and reproduction [101].

Nearly 100 different enzymes depend on zinc for their ability to catalyze vital chemical reactions. Zinc plays an important role in the structure of proteins and cell membranes [102]. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function [103]. Zinc proteins have been found to regulate gene expression [104].

2.15 COPPER

Copper is an essential trace element for humans and animals. It is found in a wide variety of foods and is most plentiful in meats, shellfish, nuts and seeds. The recommended dietary allowance is 900mcg [105]. Copper can easily accept and donate electrons and this explains its important role in oxidation-reduction reactions and the scavenging of free radicals [105].

Copper is a critical functional component of a number of essential enzymes, known as cuproenzymes. The copper-dependent enzyme, cytochrome *c* oxidase plays a critical role in cellular energy production [106]. Another cuproenzyme, lysyl oxidase is required for the cross-linking of collagen and elastin, which are essential for the formation of strong and flexible connective tissue [107].

A number of reactions essential to normal function of the brain and nervous system are catalyzed by cuproenzymes, eg neurotransmitter synthesis, metabolism of neurotransmitters, formation and maintenance of myelin and also melanin formation in the skin [100]. Clinically evident or frank copper deficiency is very uncommon and occurs under unusual conditions such as patients receiving total parenteral nutrition [107].

2.16 VITAMIN E

The term vitamin E describes a family of eight antioxidants; four tocopherols, alpha (α), beta (β), gamma (γ) and delta (δ) and four tocotrienols (also α, β, γ, and δ). α-Tocopherol is the only form of vitamin E that is actively maintained in the human body and is therefore the form of vitamin E found in the largest quantities in the blood and tissue [108]. Because α-tocopherol is the form of vitamin E that appears to have

the greatest nutritional significance, it will be the primary topic of the following discussion. Major sources of a-tocopherol in the diet include vegetable oils (olive, sunflower, safflower oils), nuts, whole grains and green leafy vegetables. The recommended dietary allowance is15mg/day [100].

The main function of α-tocopherol in humans appears to be as an antioxidant. Fats, which are an integral part of all cell membranes, are vulnerable to destruction through oxidation by free radicals. The fat-soluble vitamin, α-tocopherol, is uniquely suited to intercepting free radicals and preventing a chain reaction of lipid destruction. Aside from maintaining the integrity of cell membranes throughout the body, α-tocopherol also protects the fats in low density lipoproteins (LDLs) from oxidation. Oxidized LDLs have been implicated in the development of cardiovascular diseases. When a molecule of α-tocopherol neutralizes a free radical, it is altered in such a way that its antioxidant capacity is lost. However, other antioxidants, such as vitamin C, are capable of regenerating the antioxidant capacity of α-tocopherol [109].

Vitamin E deficiency has been observed in individuals with severe malnutrition, genetic defects affecting the α-tocopherol transfer protein and fat malabsorption syndromes [110]. Severe vitamin E deficiency results mainly in neurological symptoms such as impaired balance and coordination and muscle weakness. The developing nervous system appears to be especially vulnerable to vitamin E deficiency as seen in children with severe vitamin E deficiency from birth, who are not treated with vitamin E and who develop neurological symptoms rapidly [110]. In contrast, individuals who develop malabsorption of vitamin E in adulthood may not develop neurological symptoms for 10-20 years. Interestingly however

symptomatic vitamin E deficiency in healthy individuals who consume diets low in vitamin E has never been reported [110].

2.17 VITAMIN C

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin, which is essential for normal functioning of the body. Unlike most mammals, humans do not have the ability to make their own vitamin C. We must therefore obtain vitamin C through our diet. The main sources of Vitamin C are citrus fruits and fresh vegetables. The recommended dietary allowance is 90mg/day [100].

Vitamin C is required for the synthesis of collagen, an important structural component of blood vessels, tendons, ligaments and bone [111]. Recent research also suggests that vitamin C is involved in the metabolism of cholesterol to bile acids, which may have implications for blood cholesterol levels and the incidence of gallstones [112].

Vitamin C is also a highly effective antioxidant. Even in small amounts vitamin C can protect essential molecules in the body, such as proteins, lipids, carbohydrates and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species. Vitamin C may also be involved in the regeneration of other antioxidants such as vitamin E [111].

Severe vitamin C deficiency has been known for many centuries as the potentially fatal disease, scurvy. Symptoms of scurvy include bleeding and bruising easily, hair and tooth loss, joint pain and swelling. Such symptoms appear to be related to the weakening of blood vessels, connective tissue and bone, which contain collagen [112].

2.18 VITAMIN A

Vitamin A is a generic term for a large number of related compounds. Retinol and retinal are often referred to as preformed vitamin A [113]. The main dietary sources of Vitamin A are Cod liver oil and dairy products. The recommended dietary allowance is 900mcg/day. Inadequate retinol available to the retina results in impaired dark adaptation, known as "night blindness" [114].

Vitamin A is commonly known as the 'anti-infective vitamin' because it is required for normal functioning of the immune system [115]. Vitamin A deficiency can thus be considered a nutritionally acquired immunodeficiency disease [116].

2.19 NEOPLASTIC PROGRESSION

Neoplastic progression in Barrett's oesophagus shows a multistep progression from normal squamous mucosa to Barrett's metaplasia, to indefinite or low-grade dysplasia, to high-grade dysplasia and finally to invasive carcinoma [117]. If there are high levels of cellular proliferation in Barrett's oesophagus this is associated with a high risk of progression to adenocarcinoma [118-120].

2.20 THE CELL CYCLE

Every dividing cell passes through a cell cycle. This is an ordered set of events, culminating in cell growth and division into two daughter cells. Non-dividing cells are not considered to be in the cell cycle. The stages shown in figure two on page 36 are G1, S, G2, M. G1 stands for GAP 1. This is for growth and preparation of chromosomes for replication. The S stage stands for Synthesis. This is the stage when DNA replication occurs. The G2 stage stands for GAP 2. This is preparation for

mitosis. M stands for mitosis when nuclear and cytoplasmic division occurs [121]. (Fig 2). Abnormal accumulation of cells in the S phase (>7%) and in the G_2 phase (>6%) reflects abnormally high proliferative fractions [122].

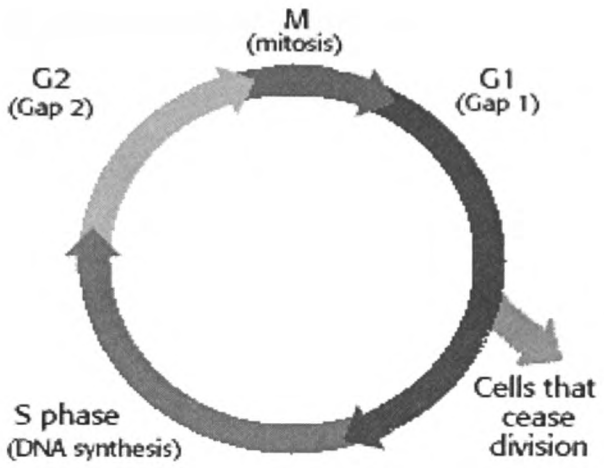

Figure Two. The cell cycle. [121]

2.21 ANTIOXIDANTS IN BARRETT'S

A recent study (2000) measured the cell proliferation in biopsies of Barrett's tissue [123]. In this study elevated proportions in the S and G_2 phases predict progression to adenocarcinoma. The percentage of cells in the G_2 phase is negatively associated with dietary selenium. Selenium from breads and grains is negatively associated with the percentage of cells in the S phase and in the G_2 phase. This suggests that decreasing dietary selenium intake and serum levels may increase the risk of progression of Barrett's oesophagus to adenocarcinoma.

In a study from Korea, Lee *et al* induced reflux oesophagitis, both acute and chronic in rats. DA-9601, a drug possessing antioxidative properties was administered to the rats. It was found that DA-9601, significantly attenuated the gross and

histopathologic scores of acute reflux oesophagitis in a dose dependent manner compared to those treated with Ranitidine (an acid suppression drug) alone. It was also found that D-9601 prevented the development of Barrett's oesophagus in the chronic reflux oesophagitis model ($p<0.05$) [124].

Rudolph *et al* [125] evaluated serum selenium levels in patients with Barrett's oesophagus enrolled in a surveillance programme. The authors found that low selenium levels were associated with an increased risk of progression to high-grade dysplasia and loss of wild type p53. It was also found that higher serum selenium levels may be associated with a reduced risk of oesophageal adenocarcinoma among persons with Barrett's oesophagus.

The most recent study (2004) is published by Fountoulakis *et al* [15]. Blood samples and endoscopic biopsies (squamous, Barrett's, and gastric) were obtained from 48 Barrett's patients, while 48 age and sex-matched controls provided blood samples only. Plasma concentrations of Vitamins A, C, and E were measured in all subjects, while Vitamin C was measured in relation to the type of mucosa. Plasma total Vitamin C level was lower in Barrett's patients compared to controls ($p=0.014$). Tissue levels of total Vitamin C were significantly lower in Barrett's compared with squamous mucosa ($p=0.047$). The authors concluded the lower levels of Vitamin C in plasma of Barrett's patients and in Barrett's mucosa compared with squamous mucosa are consistent with oxidative stress being of importance in the pathogenesis and neoplastic progression of Barrett's esophagus. There is a paucity of information in the literature relating to antioxidants and Barrett's oesophagus, and a literature search has not found any published study to challenge the above evidence.

2.22 NUTRITION AND CANCER

There have been many studies looking at the risk factors for developing cancer of the oesophagus and a large number of these have looked at the role of diet. The first indication that nutritional deficiencies may affect oesophageal cancer risk was provided by the association of the disease with Plummer-Vinson or Patterson-Kelly syndrome [126]. This syndrome is characterised by hypochromic anaemia and changes in the hypopharynx and upper oesophagus, which are associated with dysphagia and the development of cancer. Originally thought to be only the result of iron deficiency, other nutritional deficiencies may be important as well.

A review article from the Department of Epidemiology and Public Health in Yale Medical School analysed dietary factors and their role in the aetiology of adenocarcinomas of the oesophagus, and gastric cardia [127]. Diets high in total fat, saturated fat and cholesterol were associated with an increased risk of developing these cancers. The influence of the Mediterranean diet on the risk of cancers of the upper aerodigestive tract was assessed by Bosetti *et al* [128]. The cases included 598 cases of cancer of the oral cavity and pharynx, 304 cases with oesophageal squamous cell carcinoma, and 460 laryngeal cancer cases. A score summarising eight of the major characteristics of the Mediterranean diet was used. For all cancers considered, a reduced risk was found for increasing levels of the Mediterranean diet score, providing evidence that a defined nutritional pattern, which includes several aspects of the Mediterranean diet, favourably affects the risk of cancers of the upper aerodigestive tract. Galeone *et al* [129] investigated the role of fried foods on oral pharyngeal, and oesophageal cancers, and found that consumption of fried foods increased the risk of both cancer types. Finally Gonzalez *et al* [130], as part of the European Prospective Investigation into Cancer and Nutrition, investigated the risks

of gastric cancer and oesophageal adenocarcinoma associated with meat consumption. Oesophageal cancer was not affected, but gastric non-cardia cancer was associated with intakes of total meat, especially red meat.

2.23 ANTIOXIDANTS AND SQUAMOUS CELL CANCER

Of the studies looking at the risk factors for oesophageal cancer a very small number have mentioned antioxidants and in particular Selenium. The majority of papers available focus mainly on adenocarcinoma, and there are a few which discuss the role of antioxidants in the diet and the development of squamous cell carcinoma. In the next section papers relating to squamous cell carcinoma and adenocarcinoma will be discussed. Due to the large numbers of papers available on the subject a selection relevant to the discussion will be considered.

There is now sufficient literature to support the view that a diet rich in antioxidants will lead to a lower incidence of squamous cell cancer of the oesophagus. The first relevant paper was from the USA in1998 and assessed the dietary factors in patients with squamous cell carcinoma [3]. The findings were significant, in that protective effects were found with increased consumption of raw fruits and vegetables and the use of vitamin supplements, especially Vitamin C. Elevated risks of oesophageal cancer were associated with high versus low intake of red meat [3].

A French study compared the incidence of squamous cell cancer with diet in three different regions, each with different diet and drinking habits, [4] after adjustment for drinking and smoking, it was apparent that low consumption of fresh fish, vegetables and fruits and a high consumption of butter were associated strongly and independently with an increase in oesophageal cancer risk. Vitamins D, E, and

phosphorus were protective factors, whilst cholesterol appeared as a risk factor in cancer development [4].

In a study based in Shanghai, China, with 902 cases and 1,552 controls (after adjustment for cigarette smoking, alcohol consumption and other risk factors for squamous cell cancer) the authors found that increasing consumption of fruit, dark orange vegetables and beef /mutton was associated with statistically significant decreased trends for oesophageal cancer. In general, risks were about 40% lower amongst those in the upper versus lower quartiles of intake of these foods. Nutrient analysis revealed that increased dietary intake of protein, carotene, Vitamins C and E and riboflavin was associated with a reduced oesophageal cancer risk [5].

In a review article on the relationship between nutrition and oesophageal cancer seventy-six separate studies were reviewed. Most pertained to squamous rather than adenocarcinoma. It was found that the protective effect of fruit and vegetable consumption was supported by a large body of evidence, especially from case control studies [6]. Furthermore in a study carried out in South Carolina significantly increased risks of oesophageal cancer were associated with low intake of fruits, particularly citrus fruits and juices [7].

Other studies have not only shown that antioxidant intake is associated with risk reductions for oesophageal cancer, but also that the protective effect is stronger in patients who indulge in other risk factors for squamous cell carcinoma namely smoking and alcohol. A Swedish paper observed that the higher the intake of Vitamin C, Alpha-tocopherol and Beta-carotene the lower the risk of oesophageal cancer. This effect was most marked with beta-carotene, showing an inverse association with both histological types of oesophageal cancer, with risk reductions of 40-50% in the highest quartile versus the lowest. The inverse associations of antioxidants with

squamous cell carcinoma also appeared stronger amongst current smokers than amongst patients who have never smoked [8]. This would suggest that the inverse associations are stronger amongst subjects under higher oxidative stress and that protection is conferred through an anti-oxidative mechanism [8]. This has been further supported by a Greek study showing that the high levels of consumption of fruit and vegetables in their diet attenuated the harmful effects of smoking on the oesophagus [9].

The protective effect of antioxidants is also extended to alcohol. In a Greek population study it has been postulated that the high levels of alcohol drunk in the form of wine with meals is protected against by the high levels of fruit and vegetables taken with the alcohol, thus reducing the impact of ethanol on the oesophageal mucosa [9]. Support for these two speculative explanations is provided by the relatively low ratio values linking specified tobacco and ethanol increments to oesophageal cancer risk in their data [9].

2.24 ANTIOXIDANTS AND ADENOCARCINOMA

The results pertaining to adenocarcinoma are very much the same as for squamous cancer. There is a general agreement in the literature that the higher the intake of antioxidants in the form of fresh fruit, vegetables and supplements, the lower the risk of developing adenocarcinoma of the oesophagus.

A recent publication (2000) in the British Journal of Cancer looked at the role of diet and other socioeconomic factors, amongst women. A dietary questionnaire was used to obtain information for recent diet (3 years prior to interview) and at the age of 30 years. Consumption of fresh fruit, salad and vegetables was assessed by questions on food frequency. It was found that a high consumption of fruit was protective, as

was frequent use of salad vegetables. However these findings were not statistically significant, which the authors felt to be due to a limited sample size (74 cases) [10].

A much larger study of 483 patients, which looked only at the role of diet found that there was a statistically significant decreased risk of oesophageal adenocarcinoma associated with high intakes of poultry and fish, fruits including apples or pears, apricots, bananas, fresh peaches or nectarines, canned peaches, watermelon and citrus [11].

In 1996 the population of Greece had a mortality from oesophageal carcinoma of only 3.5/ 100 000 and is therefore a very low risk population. The role of diet was assessed, again by means of a food questionnaire, reporting the average frequency of consumption of 115 food or beverage items for the period of one year for each patient prior to the onset of their disease. The Greeks have a very specific diet and it was the purpose of this study to assess if their diet had a protective effect with respect to oesophageal malignancy. It was observed that added oils and fats-perhaps polyunsaturated fats were positively associated with adenocarcinoma, whereas vegetables, Vitamin A, Beta- Carotene, Vitamin C and fruits were negatively associated. Furthermore one of the findings in this particular study was that these associations were stronger for adenocarcinoma than for squamous cell carcinoma [12].

Another study performed in the United States, again confirmed decreased risks for oesophageal cancer being seen for consumption of fruits and vegetables, especially cruciferous vegetables such as broccoli. For consumers of raw fruits, raw vegetables and cruciferous vegetables, the risks for oesophageal adenocarcinoma for the highest intake levels were significantly reduced when compared with those for the lowest categories [13].

A 1997 paper by Gammon *et al* [131], which specifically focussed on tobacco, alcohol and socio-economic status, with respect to oesophageal adenocarcinoma found that although the incidence of adenocarcinoma was not affected by alcohol drinking in general there was a 40% decrease associated with wine drinking. It was postulated that there may be a protective ingredient in wine. It was also suggested that this trend may have been a result of sampling bias, as the consumption of wine in their population-based control subjects was higher and also higher amongst those who were male, white and younger in age and who reported a higher income, education and intake of beer and liquor.

2.25 SELENIUM AND CANCER RISK

Selenium has been proven to reduce cancer risk for many different types of cancer. The blood serum selenium levels have been measured in healthy subjects in six districts in the province of Merida, Venezuela [132]. There were either high or low serum-selenium containing districts. On the average a reduction of cancer incidence was observed in the districts with high serum selenium contents and in all cases evaluated, lower serum selenium values were observed in lung, stomach, hepatic, miscellaneous and total cancer patients as compared with the mean value for healthy individuals.

Furthermore, some of the most recent publications relate to the Nutritional Prevention of Cancer Trial. This was a randomised clinical trial designed to evaluate the efficacy of selenium as selenized yeast (200mcg daily) in preventing the recurrence of non-melanoma skin cancer among 1312 residents of the Eastern United States. Patients with histories of basal/squamous-cell carcinoma of the skin were assigned randomly in double-blind fashion to daily oral supplements of either

selenium, or a low selenium placebo. The primary end-points were the incidences of basal cell or squamous cell carcinomas of the skin. Secondary end-points were all-cause mortality, total cancer mortality, total cancer incidence and the incidences of lung, prostate and colorectal cancers. Selenium treatment did not significantly affect the incidence of basal or squamous cell carcinoma of the skin. There were 377 new cases of basal cell carcinoma in the selenium group and 350 cases in the control group. There were significant reductions in total cancer mortality (29 deaths in the selenium group and 57 deaths in controls) and total cancer incidence (77 cancers in the selenium group and 119 in controls) [133]. Original secondary analyses showed striking inverse associations between treatment and the incidence of total cancer numbers, lung, prostate and colorectal cancer and total cancer mortality. The protective effect of selenium was noted to be confined to males and was most pronounced in former smokers. The protective effect was also restricted to those patients with lower baseline plasma selenium concentrations [134]. An update was published in 2002, with a further 3 years follow-up of the 1312 participants in the nutritional prevention of cancer trial. The authors found that selenium supplementation did not significantly decrease lung cancer incidence in the population, but did significantly decrease the incidence amongst individuals with low baseline selenium concentrations [135].

A Finnish study looking specifically at the effect of selenium on lung cancer found a significant inverse association between serum selenium and subsequent cancer occurrence. It was also suggested that there was a stronger inverse association between serum selenium and lung cancer risk in the subpopulation of individuals with low serum levels of alpha-tocopherol (Vitamin E) [136]. This supports the theory that vitamin E reduces the oxidative damage seen in selenium deficiency [137]. This

finding has been supported by a very similar study, again from Finland, which looked at toenail selenium concentration, in male smokers only. Low selenium status was associated with an increased risk of lung cancer [138].

Evidence exists for an inverse association between selenium and bladder cancer risk. In the Netherlands Cohort study selenium levels were measured in toenail clippings in patients suffering with bladder cancer, a subcohort of 3,500 subjects was randomly sampled from the main cohort of over 100,000 subjects. Toenail clippings were provided by 438 patients with bladder cancer and 2569 controls. It was demonstrated that there was a small inverse association between toenail selenium and bladder cancer risk ($p< 0.01$). This was largely limited to the highest three quintiles of the toenail selenium distribution. The association was most pronounced in ex-smokers ($p<0.01$) and was independent of beta-carotene, Vitamin C and Vitamin E intake [139]. The authors concluded that the evidence is in favour of an inverse association between selenium and bladder cancer risk.

Not all data have found an association between serum selenium and the risk of cancer. A study looking at toenail selenium levels and bladder cancer risk, observed no association between toenail selenium concentrations and cancer risk among male smokers. However, there was a major limitation in this study as the toenail selenium levels changed over time due to the national agricultural soil fortification that occurred during the study period and the possibility that bladder cancer risk may be elevated at selenium concentrations lower than those observed in the study could not be excluded [140].

In a prospective study of toenail selenium levels and cancer among women no inverse association was found between selenium levels in toenails and cancer risk [141]. It was also noted that cancer and its treatment lowers the levels of selenium

and that levels are lower in current smokers, both factors should therefore be considered when reviewing the evidence above.

Selenium has also been found to lower the risk for colorectal adenomas [142]. It has been demonstrated that individuals with high plasma selenium levels are at decreased risk for colorectal adenomas. In this study an increase of 30 micrograms per litre in plasma selenium was associated with approximately 50% risk reduction of adenomatous polyps, even after adjustment for potential confounding variables, such as fat, fibre and antioxidant vitamins. In addition those individuals in the highest quartile of plasma selenium level were at much lower risk of colorectal adenomas than those in the lowest quartile.

In a multicentre study from the United States selenium deficiency has been implicated in the aetiology of prostate cancer. In this population-based, case-control study it was found that serum selenium was inversely associated with the risk of prostate cancer. Consistent with other studies, the inverse association was strongest among men with low serum alpha-tocopherol (Vit E) concentrations [143].

Another interesting and relevant study has been published from China measuring the effect of nutritional intervention in a region with epidemic rates of squamous oesophageal and adenomatous gastric cardia cancers [14]. Serum selenium levels were measured pre-trial and subjects were given selenium, beta-carotene and Vitamin E supplements. Selenium levels were then measured in 590 case subjects with oesophageal cancer, 402 with gastric cardia cancers and 87 with gastric non-cardia cancers as well as in 1062 control subjects. It was found that there were highly significant inverse associations of serum selenium levels with the incidence of oesophageal and gastric cardia cancers. Participants receiving supplements had a 42% reduction in oesophageal cancer prevalence. Conversely, the population proportion of

these cancers that was attributable to low selenium levels was 26.4%. Lastly there was no evidence for a gradient of serum selenium associated with the incidence of gastric non-cardia cancer.

In India, reverse smoking of chutta-rolled tobacco leaf is common practice. A chutta is a cheroot made from rolled tobacco leaves, this is then smoked by placing the lit end of the chutta in the mouth. This practise is a well-known risk factor for cancers of the upper aerodigestive tract. A study published in 2000 looked at the impact of Vitamin A, riboflavin, zinc and selenium in subjects with and without precancerous lesions in these high risk smokers [144]. 150 subjects received micronutrients and 148 controls received a placebo. Clinically complete remission of white, red and combination pre-cancerous lesions was seen in 57% of subjects on supplements whereas only 8% on placebo showed any positive response. One of the major limitations of this study was that it was not possible to attribute cancer remission to any individual micronutrient.

2.26 THE ROLE OF SUPPLEMENTATION

It would seem from the above publications that there is some evidence to support the view that selenium may have an anticarcinogenic effect. Furthermore, epidemiologic studies show that cancer incidence is higher in areas where soil selenium is low [145]. If this is the case then the question arises as to whether there is a role for selenium as a prophylactic drug in cancer.

The double-blind, placebo-controlled, randomised cancer prevention trial published using a nutritional dose of Selenium has previously been discussed [133]. The magnitude of the apparent effects on cancer incidence and cancer mortality was unexpected, in fact the blinded phase of the trial was stopped early due to this.

During the trial there were no reported side effects of selenium, although it is possible to suffer side effects if selenium is taken in high doses [146].

Selenium has both anti-mutagenic and mutagenic properties depending on the concentration and chemical form. At physiological levels found in blood, selenium has an anti-mutagenic property, but at greater concentrations mutagenicity has been observed [146]. It is not known if large amounts of selenium consumption can cause cancer in humans. A critical review of the afore mentioned study suggested that the lack of an effect on the primary endpoint (skin cancer) and the significant protection from the secondary endpoints is confusing. The authors suggest that similarly designed studies should be repeated with lung, prostate and colorectal cancer as the primary endpoints. It has been suggested that normalisation of selenium status through selenium repletion can protect high-risk groups from cancer. [147].

2.27 ANTIOXIDANTS AND CANCER RISK

The evidence for the cancer preventing properties of other antioxidants is not as clear-cut as that for selenium. The major body of evidence for selenium points towards there being anti-cancer properties. The data with reference to other antioxidants are equivocal. The evidence available supporting the anti-cancer properties of antioxidants excluding selenium has also been considered.

A paper from Texas looked at the role of isotretinoin (Vit A) in the prevention of both recurrent and secondary head and neck cancers. Patients who were disease free after primary treatment of squamous-cell cancers were randomly allocated to receive either isotretinoin or placebo taken daily for twelve months. There were no significant differences between the two groups in the number of recurrences, but the isotretinoin group had significantly fewer second primary tumours [148].

There have been several studies, which have assessed the role of antioxidants and the development of lung cancer. An early study from the USA involved supplementing the diet of 73 men with a history of 20 or more pack-years of cigarette smoking with either placebo, or folate and Vitamin B12. All the subjects were known to have bronchial squamous metaplasia. Direct cytological comparison of the two groups after four months showed significantly greater reduction of atypia in the supplemented group. This was evidence that atypical bronchial squamous metaplasia may be reduced by supplementation with folate and Vitamin B12. The authors did admit that significance of the findings was tempered by substantial spontaneous variation in sputum cytologies, the small study population and the short duration of the trial [149].

An Italian study explored the possibility of using Vitamin A as a form of treatment for patients with non-small-cell lung cancer. Following 'curative' surgery, patients were randomly assigned to either a group prescribed retinol palmitate or a control group prescribed no treatment. Following 46 months the number of patients with either recurrence or new primary tumour was 37% in the treated arm and 48% in the control arm. A statistically significant difference ($p=0.045$) in favour of treatment was therefore observed [150].

A nested case-control study was conducted within the Alpha-Tocopherol, Beta- Carotene Cancer Prevention study cohort to test for associations between selected B-Vitamins (folate, Vitamin B6, Vitamin B12) and the incidence of lung cancer. Compared with men with the lowest Vitamin B6 concentration, men in the fifth quintile had approx one half of the risk of lung cancer. Adjusting for any of the other serum factors (folate, B12) either alone or jointly did not significantly alter these estimates [151].

There is also some evidence in favour of a role of micronutrient deficiency in the development of adenomatous polyps of the large bowel and the subsequent development of colorectal cancer. A Canadian double-blind randomised trial was designed to examine the effects of Vitamins C and E on the rate of recurrence of colorectal polyps. 200 patients free of polyps after removal of at least one were randomised to receive a supplement of Vitamin C and E or a placebo. A second colonoscopy was planned after two years of supplementation. Polyps were observed in the second colonoscopy in 41% of 70 subjects on supplementation and in 51% of 67 subjects on placebo. The findings of the investigation suggested that any reduction in the rate of polyp recurrence associated with vitamin supplementation was small and a larger study would be required to ensure that an effect of this size was not a purely chance finding [152].

A similarly designed study from Italy evaluated 209 patients. The study group was given Vitamins A, C and E and these were compared with a control group. Polyps recurred in 6% of the individuals given vitamins and 36% of untreated controls ($p<0.001$). This study suggested therefore that antioxidant vitamins could be effective in reducing the recurrence rate of adenomatous polyps [153].

Antioxidants have also been shown to have an effect on gastric and oesophageal cancer. The previously discussed nutrition intervention trial carried out in Linxian, China, also looked at other antioxidants. Subjects were given four combinations of nutrients. A. retinol and zinc. B. riboflavin and niacin. C. vit C and molybdenum and D. beta-carotene, vit E and selenium. The selenium results have been discussed above. It was found that the prevalence of gastric cancer among participants receiving retinol and zinc was 62% lower than those not receiving those supplements ($p=0.09$) [154].

In the previous selenium section the bulk of evidence available supported its anti-cancer properties. This is clearly not the case for other antioxidants, because there is a large number of studies, which have failed to demonstrate any convincing evidence of anti-cancer properties.

The first of these is a large randomised, double-blind, placebo-controlled trial of beta-carotene, in which 22,071 male subjects were enrolled. Among 11,036 randomly assigned to receive beta-carotene and 11,035 randomly assigned to receive placebo, there were virtually no early or late differences in the overall incidence of any malignant neoplasm (except non-melanoma skin cancer) or in overall mortality [155].

Patients previously treated and 'cured' from head and neck cancers remain at high risk for developing a second primary in the head and neck area. Such patients were randomly assigned to receive either etretinate, a second-generation retinoid, or a placebo for 24 months. Treatment began no later than 15 days after surgery or the commencement of radiotherapy. The 5-year survival rate and disease-free survival rate were similar in the two groups. It was concluded that etretinate did not prevent second primary tumours in patients who have been treated for squamous cell carcinoma of the oral cavity and oropharynx [156].

A trial of isotretinoin in lung cancer used as the study group smokers with dysplasia/metaplasia on biopsy. These patients were randomly assigned to receive either isotretinoin or placebo daily for six months. At the completion of the study it was concluded that isotretinoin had no effect on squamous metaplasia [157]. A further study investigating the relationship between lung cancer and etretinate carried out in Canada included smokers with at least a 15-pack year history. They were screened for sputum atypia. Patients were then randomised to receive either etretinate or placebo

daily. At the end of six months there was no difference in the degree of atypia between the two treatment groups [158].

The relationship between antioxidants and lung cancer was disproven by a large randomised, double-blind, placebo-controlled trial with a total of 29,133 male smokers. They were randomly assigned to one of four regimens: alpha-tocopherol alone, beta-carotene alone, both alpha-tocopherol and beta-carotene, or placebo. Follow up was continued for five to eight years. This study concluded that there was no evidence of an interaction between antioxidants and the development of lung cancer [159].

The relationship between lung cancer, beta-carotene and vitamin A was investigated in a study of 18, 314 smokers, former smokers and workers exposed to asbestos. Subjects were recruited and randomised to receive either beta-carotene and vitamin A, or placebo. After an average of four years of supplementation, the combination of beta-carotene and vitamin A had no benefit and may even have had an adverse effect on the incidence of lung cancer. This randomised trial was therefore prematurely stopped due to these findings [160]. Although there is a large volume of evidence available regarding antioxidants and lung cancer, research has also been conducted to demonstrate the effect of antioxidants on colorectal mucosa.

A randomised controlled trial was conducted in Lebanon, looking at the effect of beta-carotene and vitamins C and E on the incidence of colorectal adenoma. 864 patients were assigned to four treatment groups. Beta-carotene, vitamin C, vitamin E, or all three, and a placebo group. Colonoscopies were performed one year and four years after recruitment. Neither treatment appeared to be effective in any subgroup of patients or in the prevention of any subtype of polyp defined by size or location [161].

In 1994 as part of the Cancer Prevention Study, analysis was performed to look at the effect of multivitamins on colon cancer mortality. It was found that multivitamin use showed little association with colon cancer mortality [162]. Another study looked at colorectal cancer and vitamin C, E, alpha-or gamma-tocopherol, retinal, alpha-or beta-carotene, lycopene, or lutein+zeaxanthin [163]. This was done as part of the larger Alpha-tocopherol, Beta-carotene Cancer Prevention Study. Again no association was found between baseline serum antioxidant concentrations and colorectal cancer. This research also investigated the links with bladder cancer.

A smaller study done as part of the Cancer Prevention Study looked at the dietary intakes of alpha-carotene, beta-carotene, lycopene, lutein/zeaxanthin, beta-cryptoxanthin, vitamins A, E and C and folate, with respect to the risk of bladder cancer. No association was found in reducing the risk of bladder cancer amongst male smokers receiving supplementation [164].

In Huixan, China a randomised double-blind intervention trial was carried out to determine whether combined treatment with retinol, riboflavin and zinc could lower the prevalence of precencerous lesions of the oesophagus. Supplementation again did not affect the prevalence of oesophageal lesions: after one year, the prevalence of oesophagitis with or without atrophy or dysplasia was 45% in the placebo group and 49% in the vitamin/zinc group [165].

A recent study (2002) in the USA looked at the effect of supplementation on stomach cancer. The use of individual vitamin C supplements, vitamin E supplements and multivitamins among 1 045 923 adults in the Cancer Prevention Study was assessed. After adjustment for potential stomach cancer risk factors the results suggested that the use of vitamin C, vitamin E, or multivitamins did not substantially

reduce the risk of stomach cancer mortality rates in these North American populations [166].

2.28 SUMMARY

It is widely accepted that there is an increasing incidence of oesophageal adenocarcinoma in Western populations [1]. The reasons for this increase are not known although it has been postulated to be due to an increase in obesity and gastro-oesophageal reflux disease, hence leading to Barrett's [24].

There have been some published studies demonstrating a link between squamous cell carcinoma and antioxidant deficiency [3-7]. Four have assessed the dietary factors in patients with squamous cell carcinoma as compared to controls, and one is a meta-analysis. It was found that a diet rich in antioxidants had an inverse association with squamous cell carcinoma of the oesophagus.

The studies discussed pertaining to adenocarcinoma were all conducted in much the same way [8-13]. The diet of patients with adenocarcinoma was assessed by means of dietary questionnaires and /or interviews. The results were all along the same common theme. A diet rich in fresh fruit and vegetables and hence antioxidants was inversely associated with adenocarcinoma of the oesophagus.

There have been very few studies on antioxidants and Barrett's. One study measured the cell proliferation in biopsies of Barrett's tissue [123]. The findings of this study suggested that decreasing dietary selenium intake and serum levels may increase the risk of progression of Barrett's oesophagus to adenocarcinoma. More recently it was found that patients with Barrett's oesophagus had a lower serum Vitamin C level than matched controls, and that Barrett's tissue itself had lower Vitamin C levels than normal oesophageal mucosa [15].

In a study from Korea reflux oesophagitis, both acute and chronic was induced in rats. It was found that an antioxidant drug prevented the development of Barrett's oesophagus in chronic reflux oesophagitis [124]. In a further study serum selenium levels in patients with Barrett's oesophagus enrolled in a surveillance programme were measured. It was found that higher serum selenium levels may be associated with a reduced risk of oesophageal adenocarcinoma among persons with Barrett's oesophagus [125].

Apoptosis is programmed cell suicide, which can be triggered by many environmental factors, the most relevant to this discussion being radiation and cytotoxic agents [93]. If a cell suffers DNA damage secondary to environmental stresses, such as free radicals, or reactive oxygen species, and is not triggered to apoptose, that cell then has the potential to develop into a malignancy [93]. *In vivo* studies have shown a protective effect of selenium against apoptosis induced by superoxide anions [167]. Apoptosis is regulated by cellular proteins, bcl-2 which inhibits, and bax, which promotes. P53 regulates apoptosis indirectly [67]. There have been studies discussed which have addressed the possibility that the expression of bcl-2, bax, and p53 within a tumour can be used to predict the response of that tumour to chemoradiotherapy [75, 77, 82].

At the time this study was undertaken (2003) there was minimal published literature studying antioxidant levels in patients with Barrett's oesophagus. There is no study to date comparing antioxidant concentrations in patients with Barrett's oesophagus, and lesser degrees of oesophageal injury. There have been very few studies of the importance of the expression of bcl-2, bax, and p53 in oesophageal tumours, and whether this relates to, or could predict the response to neoadjuvant therapy.

2.29 THESIS AIMS

The incidence of oesophageal adenocarcinoma is increasing [1], possibly due to the rise in obesity, and hence gastroesophageal reflux disease [24]. Antioxidants are trace elements found in the diet, and they have been shown to protect the cell against free radical induced DNA damage, which can potentially lead to carcinogenesis [93]. Patients with diets deficient in antioxidants have an increased risk of progression to high-grade dysplasia in Barrett's [125] ,and increased risk of developing adenocarcinoma [11].

Part of the treatment of oesophageal carcinoma is chemoradiotherapy, which is used to trigger tumour cell apoptosis, or cell death. This can be triggered both by chemotherapeutics or radiation [67]. Apoptosis is suppressed by bcl-2, and promoted by bax [67]. It is theoretically possible to predict a tumours response to chemoradiotherapy by measuring the ratio of bcl-2 to bax within the tumour [83]. Vitamin C has *in vitro* been shown to chemosensitise oesophageal cancer cells to cisplatin and 5-fluorouracil, exerting a significantly enhanced cytotoxic effect compared to both drugs individually [168], antioxidants have been shown to participate in the apoptotic pathway of oesophageal carcinoma cells [169].

The aims of the present investigations were to;

A) Investigate the concentrations of certain antioxidants in patients with Barrett's oesophagus, and lesser grades of oesophageal injury.

B) To examine whether the expression of the apoptosis-related proteins bcl-2, bax and p53 in oesophageal tumour cells can be used to identify the patients who will have a good response to chemoradiotherapy.

2.30 HYPOTHESES

1. That patients with Barrett's oesophagus will have lower serum levels of antioxidants (Selenium, Copper, Zinc, Vitamins A, C, E, β-cryptoxanthine, and xanthophyll) than control groups.

2. That patients who have a good pathological and clinical response to neoadjuvant chemoradiotherapy will have a lower level of the apoptosis inhibiting oncogene bcl-2 in the tumour cells, and a bcl-2 to bax ratio favouring bax expression.

3. That the patients responding well to neoadjuvant therapy will have a high level of the bcl-2 inhibiting protein wild type p53.

CHAPTER THREE

STUDY ONE

ANTIOXIDANTS IN BARRETT'S OESOPHAGUS

3.1 INTRODUCTION

There are many papers in the literature demonstrating a link between oesophageal cancer and serum antioxidant deficiencies [3-14]. Far fewer have investigated the link between antioxidant deficiency and the precursor of adenocarcinoma, Barrett's oesophagus [15, 123, 125]. In order to investigate whether antioxidant deficiency arises as a result of oesophageal carcinoma or the deficiency pre-dates the development of carcinoma, this study was set up in order to determine the serum antioxidant levels of Vitamins A, C and E, trace elements Selenium, Copper and Zinc, and the Carotenoids in patients with histologically confirmed Barrett's oesophagus.

3.2 METHODOLOGY

3.2.1 SOURCE OF SERUM SAMPLES

Serum antioxidant profiles were determined for the three prospectively identified cohorts of patients outlined below, during the period May-September 2003. Full ethical approval was granted by Bro-Taf Local Research Ethics Committee (Protocol #03/5011) and the Royal Glamorgan Hospital research and development board. Each control subject provided written informed consent for venepuncture and was given an information sheet. Appendix I.

1. Patients with Barrett's oesophagus (defined by endoscopic columnar lined oesophagus and intestinal metaplasia on biopsy).
2. Patients with erosive oesophagitis on oesophagogastroduodenoscopy (OGD).
3. Patient controls with reflux symptoms, but normal endoscopic appearance.

The cohorts for each study group were identified from all patients attending for upper GI endoscopy at the endoscopy suite in the Royal Glamorgan Hospital, Llantrisant. All suitable patients were recruited prospectively.

Antioxidants may act as reverse acute phase reactants [170]. This means that patients with ongoing ill health or occult inflammatory processes may have reduced levels. Patients with ongoing illness or occult inflammation were excluded on the basis of either a raised C Reactive Protein (CRP) (>10mg/L) or hypoalbuminaemia (<32mg/L). Additional exclusion critera were the presence of gastroduodenal ulceration, a history of pancreatitis or prior foregut surgery, other than cholecystectomy. In addition any patients with endoscopic Barrett's oesophagus whose oesophageal biopsies did not histologically confirm intestinal metaplasia were excluded.

For each patient the following information was recorded: age, gender, type, and duration of symptoms and the results of the OGD. Results of any histological biopsies taken at the time of endoscopy were also recorded. These results were collected on a questionnaire proforma. Data were stored on a specifically designed database, using a Pentium III personal computer and Microsoft Access with Windows 2000 home edition.

3.2.2 SAMPLE SIZE DETERMINATION

For this study we assumed the magnitude of the difference between Barrett's and control patients would be of the order of 0.75 standard deviations. Assuming a significance concentration of 5% and a power of 80%, equal groups of 30 patients would be required.

3.2.3 BIOCHEMICAL ANALYSIS

The biochemical parameters assessed comprised plasma concentrations of the trace elements selenium, zinc, copper, Vitamin A, C and E. 15mls of blood was collected at the time of attendance for OGD. Venous blood was obtained after an overnight fast. Samples for trace element analysis were collected in trace element free sodium heparin vacutainers (Becton Dickinson, Cedex, France); those for vitamin analysis were taken into lithium heparin vacutainers (Becton Dickinson, Cedex, France) and transported to the laboratory in a light excluding container. All samples were centrifuged within 30 minutes of collection. Separated plasma was divided into aliquots and stored at the following temperatures before analysis: trace elements minus 20°C, vitamins A and E minus 20°C and vitamin C minus 70°C. Samples were stored at this temperature because vitamin C is more thermally unstable than vitamins A and E.

The samples were processed in the Department of Biochemistry, University Hospital of Wales. Zinc was measured using flame atomic absorption on an AA-10 spectrophotometer (Varian, Inc., NJ). Selenium was measured by electrothermal graphite furnace atomic absorption using a palladium nitrate matrix modifier on an AA-600 spectrophotometer (Varian, Inc., NJ). Vitamins A, C and E were ascertained by high performance liquid chromatography with spectrophotometric detection using wavelengths vitamin A: 327nm; vitamin E:292nm; vitamin C 451nm respectively. CRP and Albumin levels were ascertained the biochemistry laboratory, in the Royal Glamorgan Hospital. Both were measured by dry slide technology on a Vitros 950 analyser (Ortho-Clinical Diagnostics, Amersham, Bucks, U.K.).

3.3 RESULTS

3.3.1 THE STUDY POPULATION-THE THREE STUDY GROUPS

1. 36 patients (33 males, 3 females) with Barrett's oesophagus (defined by endoscopic columnar lined oesophagus and intestinal metaplasia on biopsy), mean age 57 years (range 39-85years).
2. 32 patients (20 males, 12 females) with erosive oesophagitis, mean age 59 years (range 35-77 years).
3. 35 patient controls (15 males, 20 females) with reflux symptoms and normal endoscopic appearances of the oesophagus, stomach and duodenum, mean age 49 years (range 20-72 years).

See chart one page 63. This graph demonstrates that there is a preponderance of males to females in the Barrett's group with a ratio of 11:1. This is at odds with the literature with a quoted M:F ratio of 1.7 for patients with Barrett's oesophagus [171]. Chart two page 63 shows the age distribution for the entire study population (n=113). The mean age of the patients in the Barrett's and oesophagitis groups was 57 and 59 whereas the mean age of the patients in the normal control group was 49.

The M:F ratio, and age difference between the groups in the present study will not have an adverse effect on the results, as a previous study of antioxidants in chronic pancreatitis demonstrated that antioxidant levels did not change between sexes or age groups [172]. Many of the previous studies discussed had not felt it necessary to age, and sex match controls, although we accept this is a possible weakness of the study.

All of the patients recruited were taking proton pump inhibitors for treatment and symptom control.

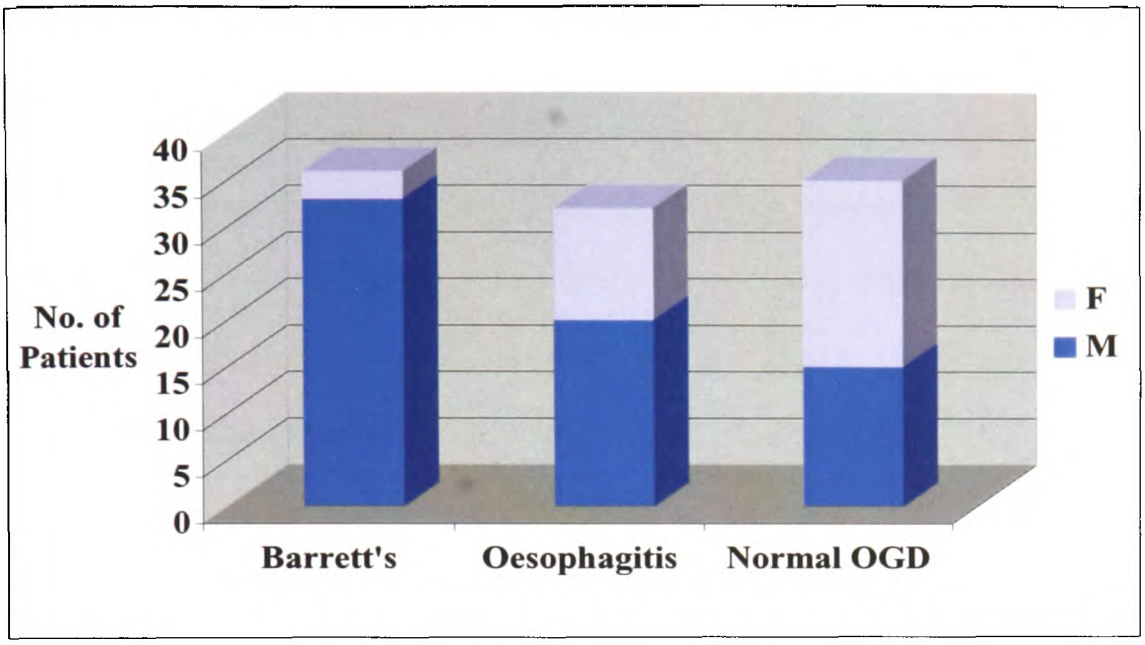

Chart one. Sex ratio by study group.

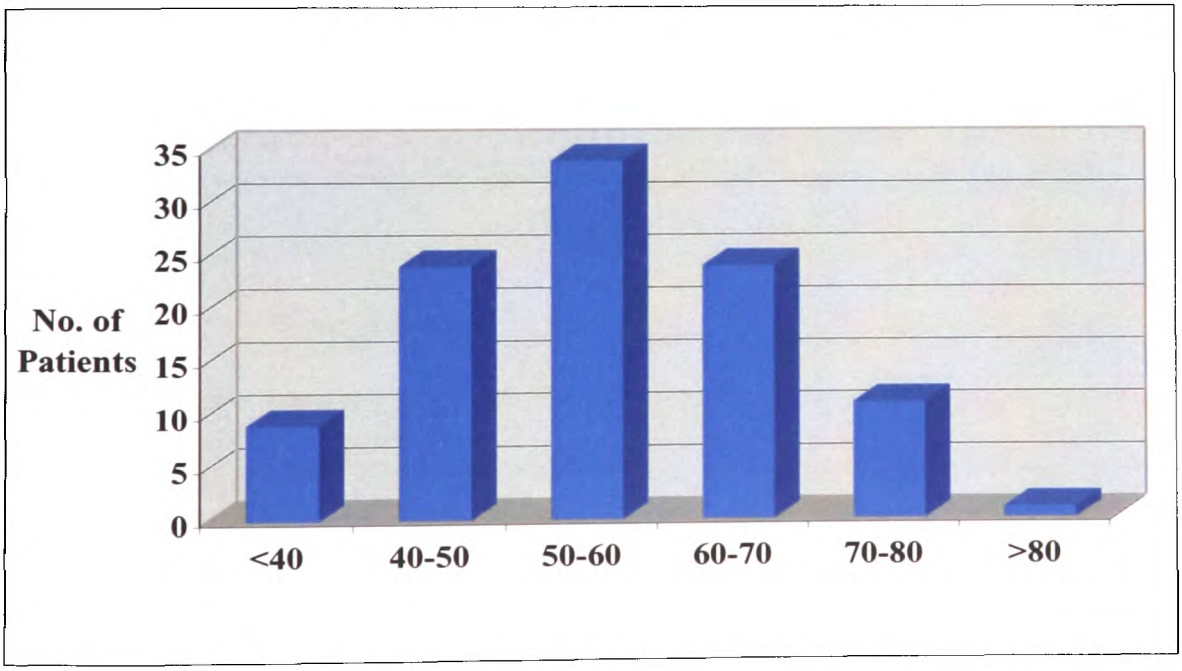

Chart two. Age ranges for entire group. n=103

3.3.2 RESULTS

There are three methods for patients presenting to the endoscopy department for oesophagogastroduodenoscopy (OGD). First is the open access list, which means that the patient can be referred directly for OGD by their general practitioner. Second is via the outpatient department, meaning that the patient is under the care of a consultant within the hospital. Thirdly patients can undergo an OGD whilst being an inpatient within the hospital.

There is a notable difference between the mode of presentation of the three study groups. They will be numbered as follows. The Barrett's group was group one, the erosive oesophagitis group was group two, and the normal control group was group three. This will hold throughout the text and for the labelling of diagrams.

The most common mode of presentation within group one was via outpatients, whilst the most common mode of presentation within groups two and three is through open access lists. See chart three on page 68. This is due to the fact that in the Royal Glamorgan Hospital most Barrett's patients were under long term follow up and automatically recalled for OGD. The reason for this is that biopsies can be taken to look for evidence of dysplasia, which is a sign of progression to adenocarcinoma. These biopsies are taken according to the Seattle protocol [173]. Using a biopsy forceps four biopsies (one per quadrant of the oesophagus) were obtained from every centimetre of the Barrett segment. In all patients, at least one control biopsy specimen was also taken from the gastric fundus and several biopsy specimens were taken from any visible mucosal abnormality. For most of the patients in groups two and three this was their first presentation and they were referred for open access OGD via their general practitioner.

The presence or absence of symptoms was ascertained for each patient. These will be discussed below as per study group. There were 36 patients in group one. Heartburn was the most common symptom with a third of the group reporting this. Regurgitation and dysphagia were also symptoms that were experienced by this group, but far fewer patients (13% and 5% respectively) had these. See chart four on page 68.

The 32 patients in group two complained of far more symptomatology than those in group one. Again heartburn was the most common, with 65% of the group experiencing this on a regular basis. Approximately one third of the group had regurgitation or epigastric pain and dysphagia was experienced by 12%. See chart five on page 69. The patients in group three most commonly had symptoms of heartburn and regurgitation, affecting over two thirds of the group. Just under one third had epigastric pain. See chart six on page 69.

The difference in symptoms can be explained by the fact that mucosa regularly exposed to acid is less sensitive to it. This has been demonstrated in a 1995 paper from Los Angeles [174]. A naso-gastric tube was passed and saline solution introduced into the oesophagus. This was then switched to hydrochloric acid without the patient being informed, and the patients symptomatic response noted for 30 minutes. Precipitation of pain by acid that was relieved by reinfusion of saline solution was a positive response. It was found that 38% of patients with no previous symptoms of reflux had a positive result as compared with 26% of patients known to have an abnormal 24-hour pH profile indicating reflux [174]. It has also been shown that some patients with severe symptoms may be found on endoscopy to have mild disease, whereas others with severe disease such as Barrett's oesophagus may have relatively few symptoms.

There was no difference in the frequency or duration of symptoms between any of the study groups. The results were therefore examined for the entire number of patients recruited in the study (n=103). The frequency of heartburn symptoms varied widely, 70% experiencing them on a daily basis, 21% on a weekly basis and 9% on a monthly basis. The duration of heartburn symptoms again varied widely, with 6 % having them for under 3 months and 16% having them for over 36 months. The largest group was 24-36 months. See chart seven on page 70.

There was a wide variation in the frequency of regurgitation symptoms. 41% reported them daily, 41% weekly and 18% monthly. There was no difference between the study groups, therefore these figures are for all patients (n=103). There was again a marked variation in duration of regurgitation symptoms. 8% had experienced symptoms for less than three months and 16% had had symptoms for over 36 months. The largest group was again 24-36 months, with around one third of all patients having regurgitation having had it for this duration of time. See chart eight on page 70.

It is noted that there was a decrease in the number of patients reporting their symptoms lasting for 18-24 months. See charts seven and eight on page 70. A likely explanation for this is that most patients first presenting to their General Practitioner will be empirically treated. If symptoms were still present at one year this is the point at which both patient and GP require a diagnostic endoscopy for clarification. In many patients this allowed a diagnosis to be made and treatment to commence with the subsequent relief of symptoms.

Just over half of the patients experiencing epigastric pain suffered it daily, one third had it weekly and one tenth monthly. 10% had had their symptoms for less than 3 months and 20% had had their symptoms for over 36 months. The largest group was

6-12 months, with just over one third of patients having their symptoms for this length of time. There was no difference between the groups.

One third of patients with dysphagia had symptoms daily and two thirds weekly. All patients had experienced symptoms for between 6 and 18 months. There was no difference in the frequency or duration between each group.

Other symptoms patients experienced were chest pain, belching and choking. There was no difference between these per study group. These symptoms were only experienced in a small proportion of all the patients.

Of the whole group of 103 patients 40% had an identifiable hiatus hernia. See chart nine on page 71. These were not found more commonly in any of the study groups. Of those with a hiatus hernia, 57% were small and 43% were large, although this was subjective for operator interpretation.

The degree of inflammation of the oesophagus in the 32 patients in group two was assessed using the Los Angeles classification [175]. This is as follows:-

Grade A. Mucosal breaks confined to the mucosal fold, each <5mm.

Grade B. At least one mucosal break >5mm confined to the mucosal fold but not continuous between folds.

Grade C. Mucosal breaks that are continuous between the tops of mucosal folds but not circumferential.

Grade D. Extensive mucosal breaks engaging at least 75% of the oesophageal circumference.

31% of these patients had grade A inflammation, 59% had grade B inflammation and 10% had grade C or D inflammation. See chart ten on page 71.

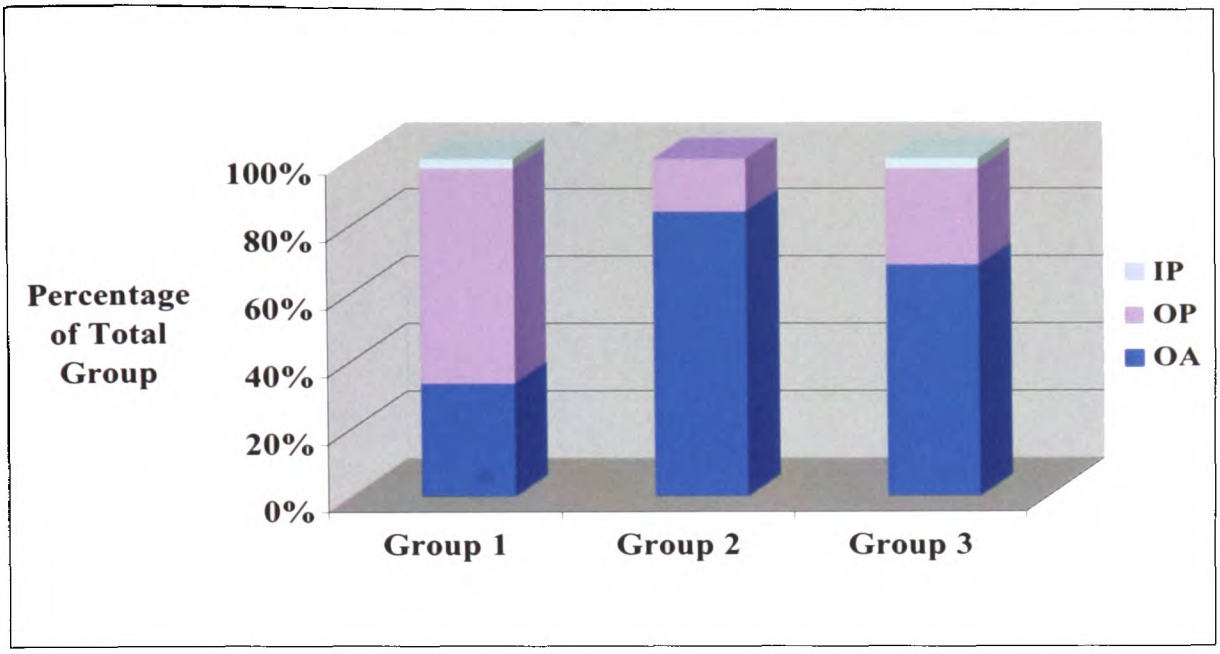

Chart three. Referral source by study group.
IP= Inpatient OP= Outpatient OA= Open Access

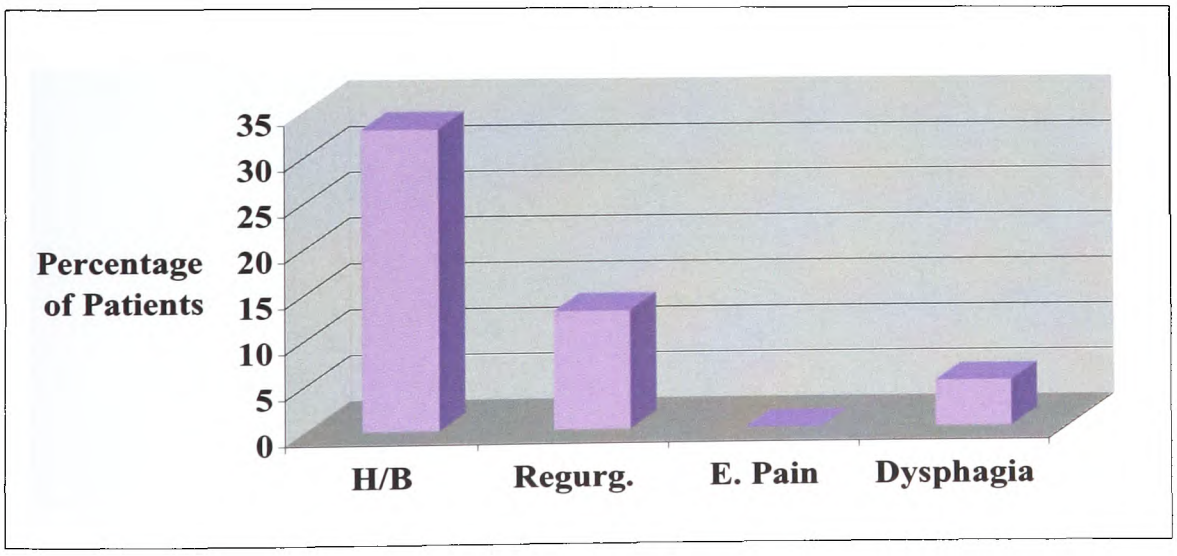

Chart four. Percentage of symptoms experienced by patients in group one. (n=36)
H/B= Heartburn Regurg.= Regurgitation E.Pain= Epigastric Pain.

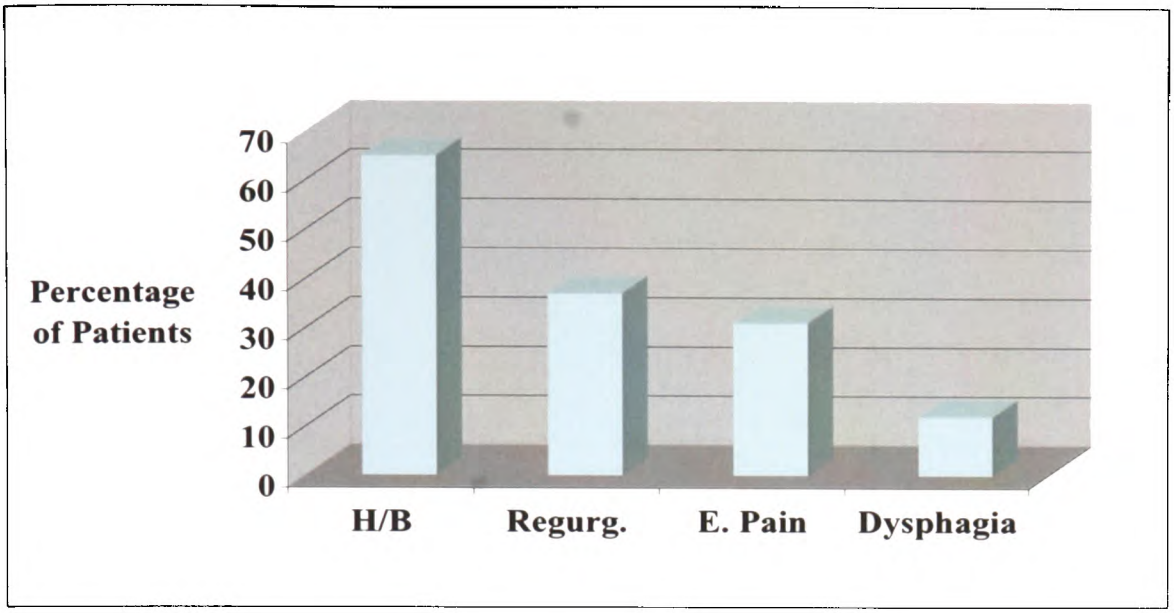

Chart five. Percentage of symptoms experienced by patients in group two. (n=32)
H/B= Heartburn Regurg.= Regurgitation E.Pain= Epigastric Pain.

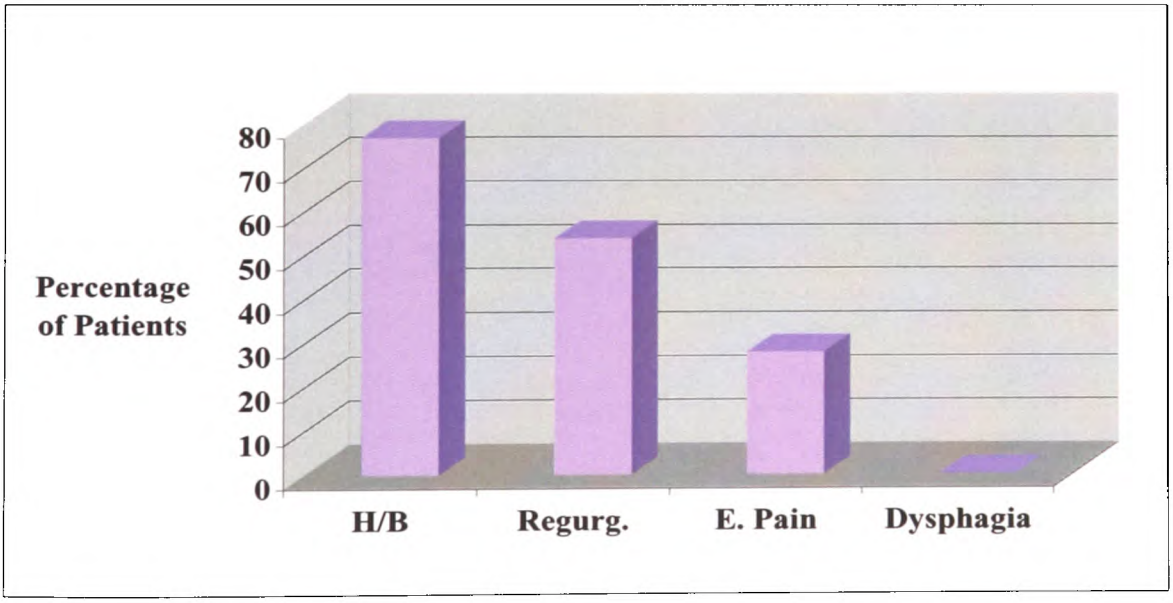

Chart six. Percentage of symptoms experienced by patients in group three. (n=35)
H/B= Heartburn Regurg.= Regurgitation E.Pain= Epigastric Pain.

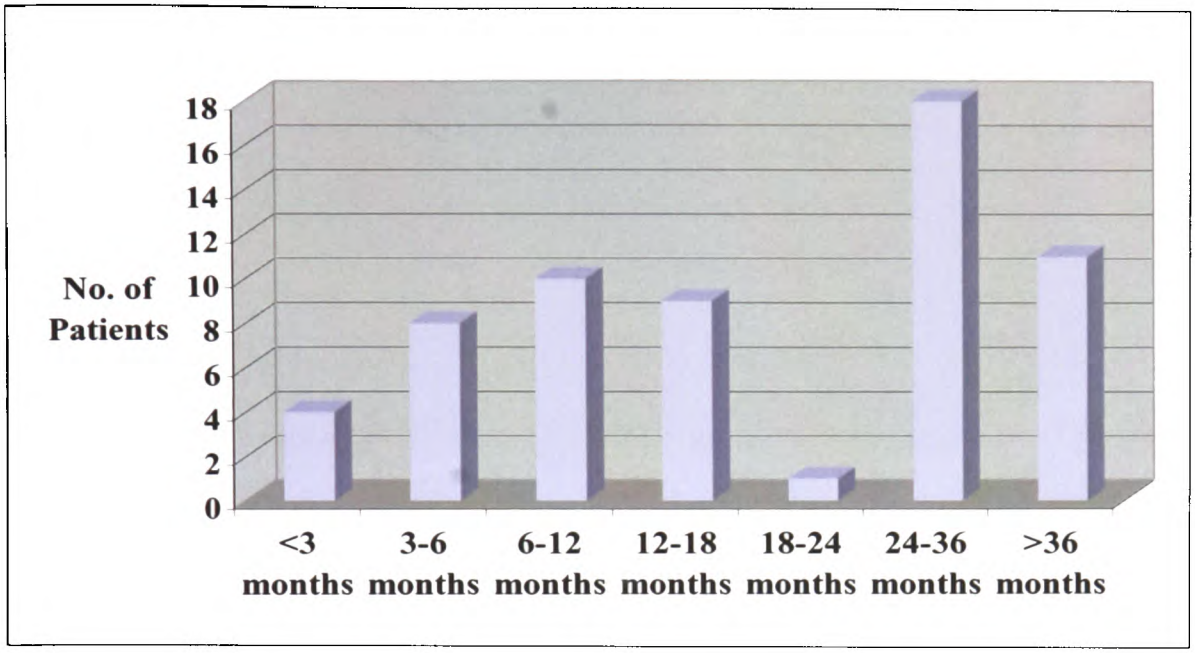

Chart seven. Duration of heartburn for all patients. (n=103)

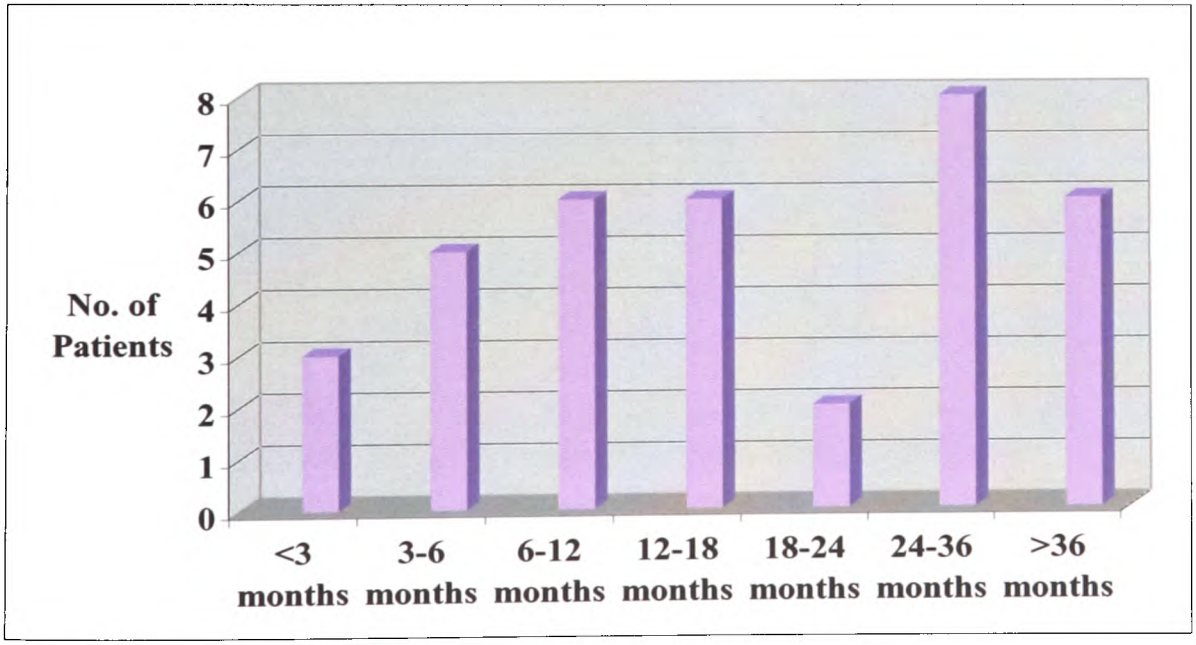

Chart eight. Duration of regurgitation for all patients. (n=103)

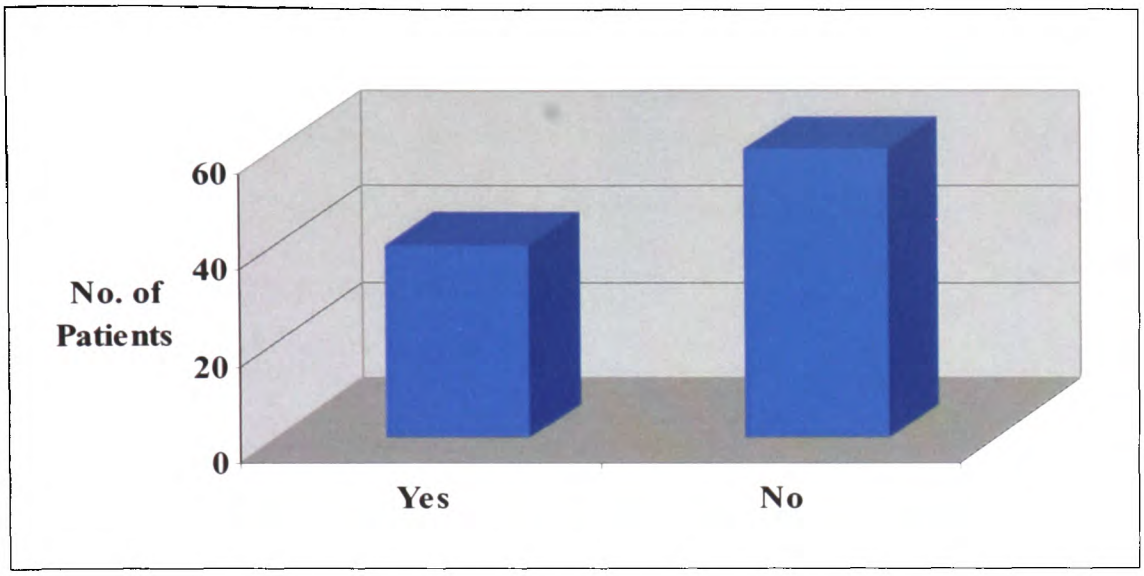

Chart nine. Presence of hiatus hernia for all patients. (n=103)

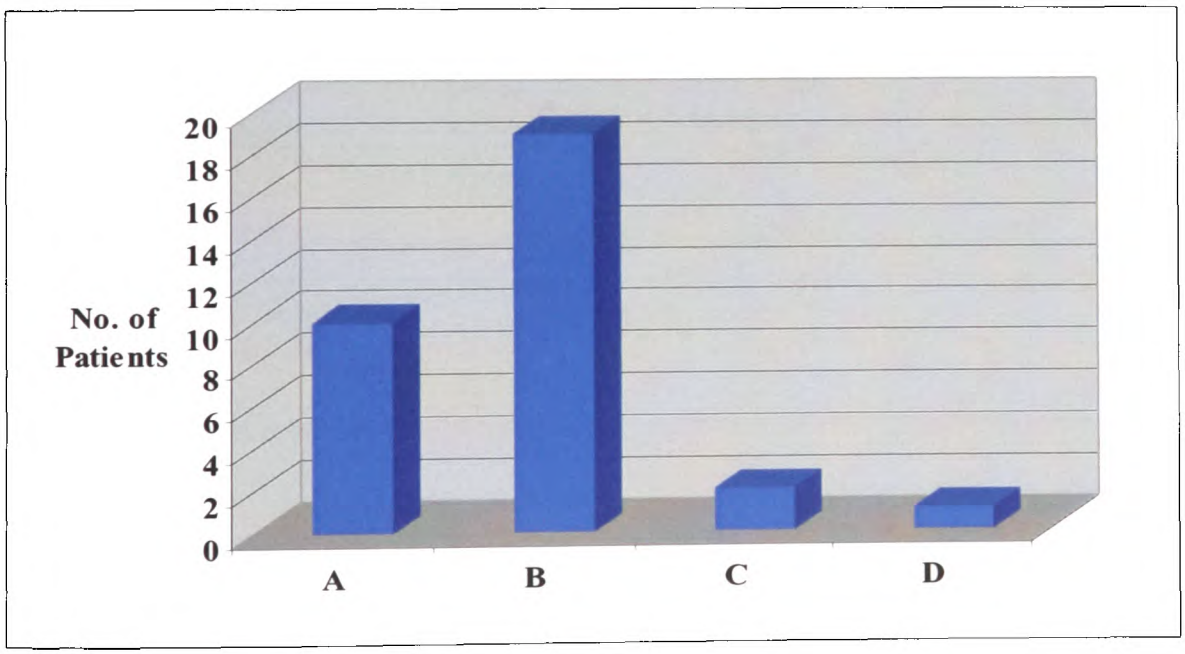

Chart ten. Los Angeles Classification of oesophagitis in group two. (n=32)

3.3.3 ANTIOXIDANTS IN BARRETT'S GROUP/CONTROLS

In this study deficiencies of four antioxidants were found in the Barrett's group as compared to one or other control group. See table one below. The serum levels of Vitamin C, β-cryptoxanthine, xanthophyll, and selenium were reduced in the Barrett's group, as compared to controls.

μmol/L	Group One (n=36)	Group Two (n=32)	Group Three (n=35)	p-value
β-cryptoxanthine Normal value 0.07-0.88	0.06 (0.05-0.08)	0.10 (0.08-0.13)	0.08 (0.07-0.10)	0.008 vs Group 2
Vitamin C Normal value 22-110	18.3 (13.2-23.4)	26.6 (19.6-33.6)	27.1 (21.4-32.7)	0.05 vs Group 2 0.02 vs Group3
Selenium Normal value 0.8-1.4	0.72 (0.67-0.78)	0.75 (0.71-0.79)	0.81 (0.74-0.87)	0.05 vs Group 3
Xanthophyll Normal value 0.4-1.00	0.35 (0.27-0.44)	0.55 (0.41-0.69)	0.48 (0.33-0.63)	0.02 vs Group 2

Table One. Results of serum Vitamin C, β-cryptoxanthine, xanthophyll, and selenium for Groups One, Two, and Three. Values shown are means, and 95% confidence intervals.

Statistical analysis was performed using the ANOVA test. This is similar to the independent t-test but allows comparison between more than two conditions. A significant p-value will indicate that there is an effect somewhere between the groups, but will not indicate where the effect lies [176]. Where the ANOVA has shown a significant p-value then follow-up t-tests on both pairs were undertaken.
There was no difference between the serum levels of the other antioxidants. See table two page 73.

μmol/L	Group One (n=36)	Group Two (n=32)	Group Three (n=35)	p-value
α-carotene Normal value 0.02-0.22	0.05 (0.04-0.06)	0.05 (0.04-0.06)	0.05 (0.04-0.06)	0.66
β-carotene Normal value 0.07-0.88	0.28 (0.24-0.32)	1.10 (0.53-2.74)	0.32 (0.27-0.37)	0.33
Lycopene Normal value 0.11-0.52	0.32 (0.26-0.38)	0.28 (0.20-0.35)	0.26 (0.21-0.31)	0.41
Vitamin A Normal value 1.1-2.6	2.17 (1.97-2.38)	2.01 (1.82-2.21)	1.97 (1.83-2.11)	0.23
Vitamin E Normal value 11-47	26.7 (23.0-30.4)	29.1 (25.6-32.6)	28.3 (25.1-31.4)	0.59
Copper Normal value 11-22	16.0 (15.0-16.9)	16.9 (15.5-18.3)	16.3 (14.9-17.7)	0.58
Zinc Normal value 8-17	13.3 (11.4-15.1)	12.9 (12.2-13.5)	13.1 (11.6-14.6)	0.93

Table Two. Serum levels of those antioxidants not reduced in either of the study groups. Values shown are means and 95% confidence intervals.

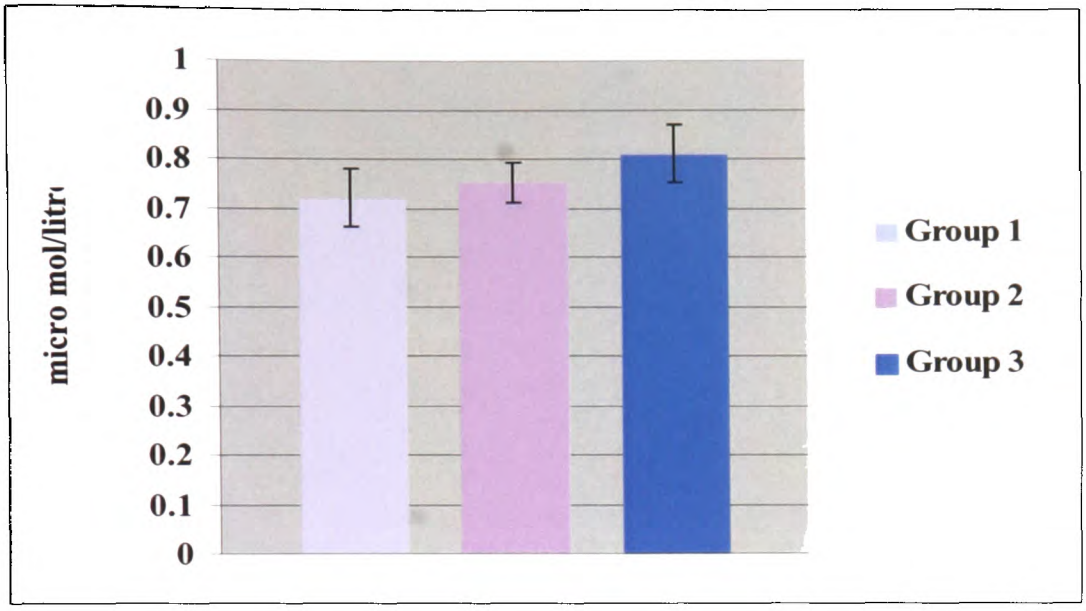

Chart 11. 95% confidence intervals for selenium levels (μmol/L) per study group.

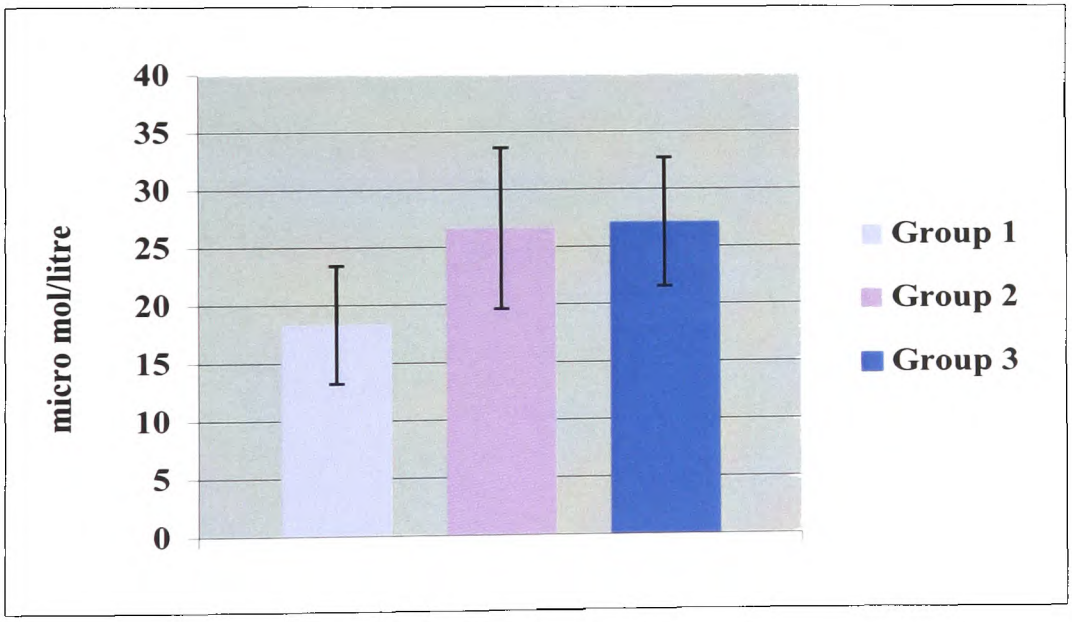

Chart 12. 95% confidence intervals for vitamin C levels (μmol/L) per study group.

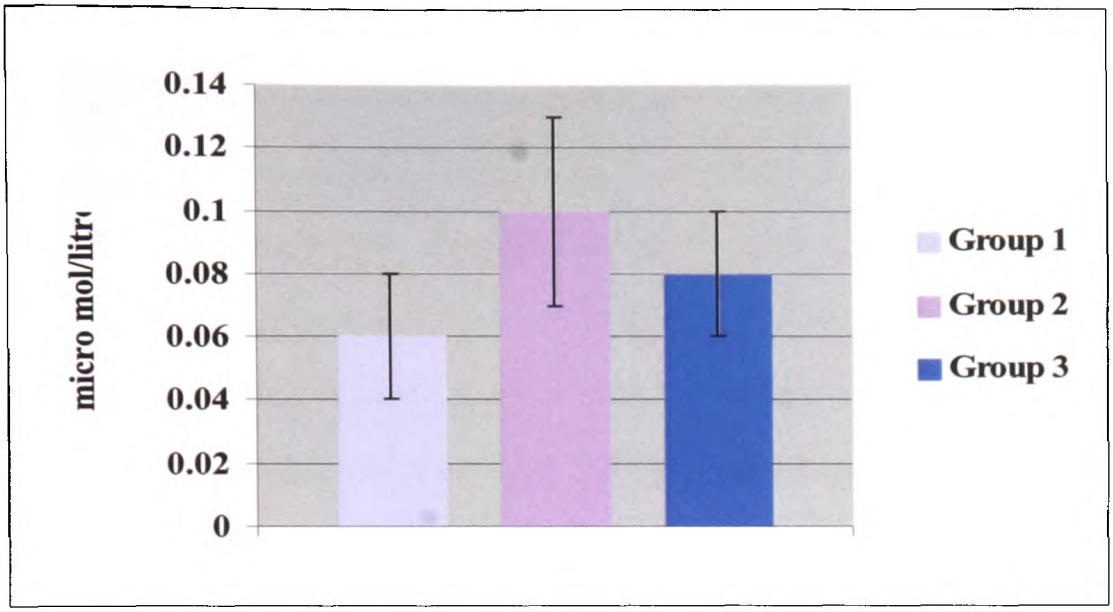

Chart 13. 95% confidence intervals for β- cryptoxanthine levels (μmol/L) per study group.

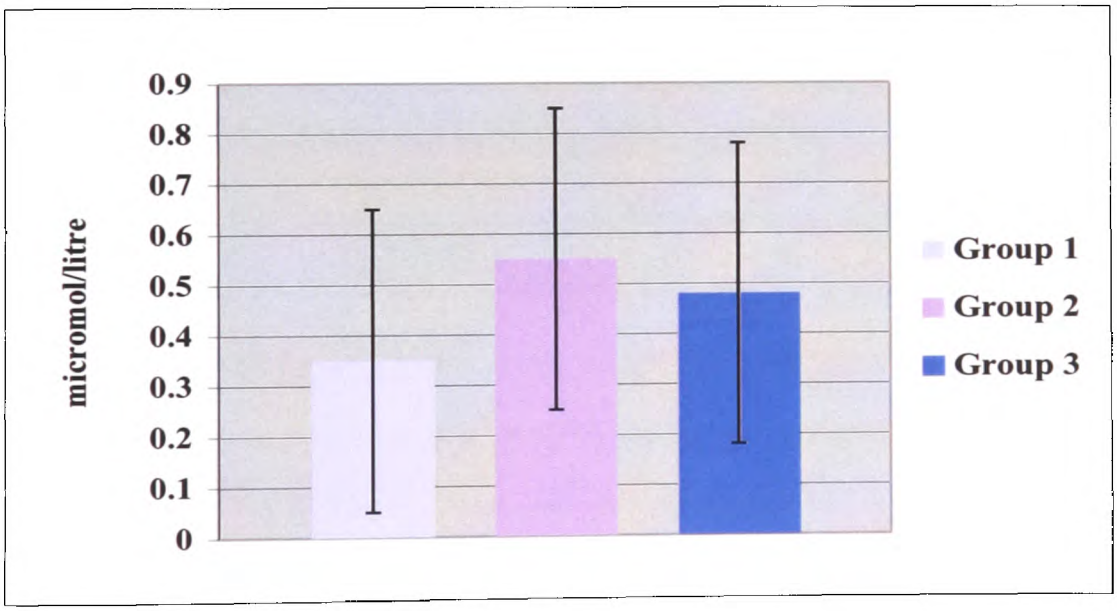

Chart 14. 95% confidence intervals for xanthophyll levels (μmol/L) per study group.

3.4 DISCUSSION

The clinical observations that were undertaken showed there is a difference in the severity of symptoms reported in the three study groups. The Barrett's group (group one) experienced far fewer symptoms than the oesophagitis group (group two) who in turn experienced less than the control group (group three). The difference in symptoms can be explained by the fact that mucosa regularly exposed to acid is less sensitive to it. This has been demonstrated in a 1995 paper from Los Angeles [174]. A naso-gastric tube was passed and saline solution introduced into the oesophagus. This was then switched to hydrochloric acid without the patient being informed, and the patients symptomatic response noted for 30 minutes. Precipitation of pain by acid that was relieved by reinfusion of saline solution was a positive response. It was found that 38% of patients with no previous symptoms of reflux had a positive result as compared with 26% of patients known to have an abnormal 24-hour pH profile indicating reflux. It has also been shown that patients with severe disease such as Barrett's oesophagus may have relatively few symptoms [174].

It was noted that there was a decrease in the number of patients reporting their symptoms lasting for 18-24 months. A likely explanation for this is that most patients first presenting to their General Practitioner with reflux symptoms will be empirically treated with proton pump inhibitors in order to reduce the amount of acid in their stomachs and hence the reflux. If symptoms are still present at one year this is the point at which both patient and GP require a diagnostic endoscopy for clarification. In many patients this will allow a diagnosis to be made and appropriate treatment to commence with the subsequent relief of symptoms.

Our main findings were that patients with Barrett's oesophagus had significantly lower serum concentrations of the antioxidants vitamin C, β-cryptoxanthine, selenium, and xanthophylls compared to patient controls with little or no oesophageal injury.

A dietary history was not taken as part of this study. There have been many dietary inventory studies published in the literature, which have been discussed previously in this work, and it was not felt necessary to replicate that research. On the basis of these results it is highly likely that the subjects in our study found to have decreased serum levels of antioxidants have a poor intake of antioxidants in the form of fresh fruit and vegetables in their diet. Two of the three studies quoted in this research designed to examine the links between antioxidants and Barrett's have taken dietary information into account, and indeed have proven a direct relationship between the dietary content and serum levels of antioxidants [15, 123]. Our study was designed to investigate whether patients with Barrett's have low serum antioxidant levels, as compared to controls with both reflux induced oesophageal damage, and normal oesophageal mucosa.

Antioxidants may act as reverse acute phase reactants [170]. This means that patients with ongoing ill health or occult inflammatory processes will have a higher turnover of antioxidants hence resulting in reduced serum levels. So as to ensure our results were not biased by including patients with occult illness or inflammation these subjects were excluded on the basis of either a raised C Reactive Protein (CRP) (>10mg/L) or hypoalbuminaemia (<32mg/L).

There are two possible explanations for our findings. The first is that patients developing Barrett's oesophagus also develop a concurrent deficiency in serum antioxidants. A second and more likely explanation in the light of the previously

discussed dietary inventory studies is that patients with a pre-existing antioxidant deficiency develop Barrett's oesophagus and in turn are at risk of adenocarcinoma.

It is postulated that Barrett's develops as a result of reflux disease, in which the injured squamous epithelium heals through a metaplastic process. This results in an abnormal columnar epithelium lining the oesophagus [26]. As antioxidant deficiency results in the increased processes responsible for the development of malignancies [94] and Barrett's oesophagus is a pre-malignant condition [33] it is possible that the antioxidant deficient patient is unable to protect the epithelium from this process and is therefore more likely to develop Barrett's oesophagus as a consequence of their reflux.

At present there is a relative paucity of information in the literature relating to antioxidants and Barrett's oesophagus. Two studies from one institution (of patients with Barrett's oesophagus) identified low levels of selenium in Barrett's patients who progressed onto adenocarcinoma [123, 125]. Rudolph *et al* [125] evaluated selenium levels in patients with Barrett's oesophagus enrolled in a surveillance programme. The authors found that low selenium levels were associated with an increased risk of progression to high-grade dysplasia. Thus these two studies support the hypothesis that antioxidant deficiency pre-dates and possibly predisposes to the development of Barrett's and hence adenocarcinoma.

Moe *et al* [123] found that a decreased dietary selenium intake resulting in decreased serum levels increased the risk of progression of Barrett's oesophagus to adenocarcinoma. A number of studies have evaluated retrospectively antioxidant intake in patients with oesophageal adenocarcinoma [10-14]. These reports suggested a lower incidence of oesophageal adenocarcinoma for diets rich in citrus fruits, certain vegetables and several antioxidants, notably Vitamin C and β-Carotene, therefore

suggesting a protective effect of such a diet. These results show that patients with a poor antioxidant diet have developed adenocarcinoma. This would further support our hypothesis that the antioxidant deficient patient is at a higher risk of developing Barrett's oesophagus as a result of reflux disease.

The present results add support to the thesis that antioxidants protect the oesophageal mucosa from metaplastic change in response to reflux. It is possible that elevated serum antioxidant levels protect mucosa inflamed by reflux from DNA damage, metaplastic change, and the subsequent development of Barrett's oesophagus.

Is there any therapeutic potential for our observations? For example should antioxidant supplementation be recommended in order to prevent the development of Barrett's oesophagus and hence adenocarcinoma? Previously discussed epidemiologic studies have shown that cancer incidence is higher in areas where soil selenium is low [145]. Furthermore the epidemiology of squamous carcinoma in high incidence regions of China supports an association with antioxidant deficiency [177], as does the epidemiology of adenocarcinoma in China [14]. Lastly, large scale antioxidant supplementation studies have demonstrated a reduction in the risk of malignant transformation of squamous dysplasia [154]. It is an appealing concept that the same will hold true for glandular dysplasia and adenocarcinoma, although larger scale research on the subject is necessary. Whilst it was an attractive proposition to carry out supplementation of antioxidants in this study, it was unfortunately not deemed possible due to limitations of time, funding and resources.

3.5 CONCLUSION

1. Poor nutrition and the resulting antioxidant deficiencies are a risk factor in developing metaplastic change of the oesophageal mucosa in response to acid reflux.

CHAPTER FOUR

STUDY TWO

bcl-2, bax AND p53 IN OESOPHAGEAL TUMOURS

4.1 INTRODUCTION

Patients receiving neoadjuvant chemoradiotherapy for treatment of oesophageal carcinoma are divided into two groups. A proportion of patients will respond to the treatment, whilst others will not. An elevated ratio of bcl-2 to bax in prostate cancer has been shown to predict an increased risk of the cancer failing to respond to radiotherapy [82]. Over-expression of p53 has been shown to decrease the response to chemoradiotherapy in oesophageal carcinoma [75], and forced expression of the wild-type p53 gene has been shown to increase the sensitivity to chemoradiotherapy [77].

This study was designed to determine whether a tumours response to chemoradiotherapy can be predicted by expression of bcl-2, bax and p53. Chemoradiotherapy is associated with significant side effects [45-47]. It was also of interest whether the patients whose tumour had a good response to chemoradiotherapy would have more sensitive normal tissues. This would manifest in increased post-operative complications because of increased lung and cardiac damage from the treatment. The post-operative complications were therefore compared to the patients' response to chemoradiotherapy.

4.2 METHODOLOGY

4.2.1 COLLECTION OF SAMPLES

Full ethical approval was granted by the Bro-Taf Local Research and ethics committee (Protocol #03/5133) before beginning the project. The study group comprised of patients having neoadjuvant chemoradiotherapy prior to a planned oesophagectomy. There were twelve patients in the study group. A power sample test was not carried out for several reasons. There are no known results on which to base

such a calculation, in addition to this the results are not expressed numerically but in terms of low, moderate or high expression. This was corroborated by the fact that none of the papers I have quoted had determined a sample size. Finally we were only able to obtain funding for a limited number of slides to be processed. This study therefore must be viewed strictly as a pilot study.

Ten of the subjects had proven adenocarcinoma and two had proven squamous cell carcinoma of the oesophagus. The diagnosis was confirmed by histological examination of biopsies taken at the time of endoscopy. If a clinically suspicious area is seen in the oesophagus or stomach at the time of endoscopy then multiple samples of this are taken with biopsy forceps passed down a channel of the endoscope for this purpose. These samples are then examined by an experienced pathologist. There were no data available as to the sensitivity and specificity of these biopsies. It is certainly approaching 100%, as the clinical implications of a false positive or negative are enormous. Sample sizes of n=1 are treated and offered major surgery in the form of an oesophagectomy in surgical practice on a daily basis on the results of these biopsies. There is scope for a false negative result to occur, this is due to the tumour itself not being biopsied, as often there is inflammatory tissue associated with it. If this occurs, and there is a strong clinical suspicion the endoscopy, and biopsies are repeated.

Data regarding demographics were obtained from the Royal Glamorgan Hospital database. Clinical and pathological reports were similarly obtained. Three cycles of cisplatin and 5-fluorouracil together with concurrent radiotherapy were administered in Velindre hospital. The original biopsy specimens from the tumours were obtained from the department of pathology of the hospital in which the tumour was diagnosed, and taken to the University Hospital of Wales where the immunohistocytochemistry analysis was performed.

4.2.2 IMMUNOHISTOCYTOCHEMISTRY ANALYSIS

All analysis was carried out in the department of Pathology at the University Hospital of Wales. All slides were stained on Autostainer (Dakocytomation). Mouse monoclonal antihuman bcl-2 antibody was used at 1/100 dilution Code M0887 (Dakocytomation). DO7 monoclonal antibody against p53 protein was used at 1/200 dilution Code M 7001 (Dakocytomation). The slides were blocked for endogenous biotin (Vector lab SP-2001), and endogenous peroxidase (Dakocytomation). The antigen retrieval protocol was 600ml of 10 mmol EDTA pH 7.0, 20 minutes of microwave at 800 watts with five minutes standing time.

Formalin fixed paraffin processed sections were cut at four microns and floated onto superfrost plus electrostatically charged slides. These were then heated at 60° Centigrade overnight. Sections were dewaxed in Xylene, dehydrated in alcohol and placed in running tap water. The slides were then placed into antigen retrieval solution and microwaved in a covered container, flushed with running tap water and placed into deionised water. They were placed into a wash buffer for five minutes before being placed onto the autostainer. Immunoperoxidase slides were counterstained with Haematoxylin, dehydrated in alcohol, cleared in xylene and mounted with a coverslip.

An Olympus microscope model BH-2 was used to examine the slides. Firstly there were two control slides. The purpose of these was to demonstrate a positive result for bcl-2 and p53. For bcl-2 a slide of tonsil was used, and for p53 a slide of colorectal cancer was used. Tonsillar tissue has a high expression of bcl-2 and colorectal cancer has a high expression of p53. [178]

Wild-type p53 protein mediates apoptosis by directly activating the expression of bax and indirectly inhibiting the expression of bcl-2 [69, 70]. If the p53 is mutated

this effect will be lost. Forced expression of wild-type p53 has been shown to increase the sensitivity to chemoradiotherapy [77]. Therefore if the tumour has a higher expression of wild-type p53 it may be expected to be more sensitive to chemoradiation. Wild-type p53 is not demonstrated with immunohistocytochemistry, whereas mutated p53 is. Therefore the higher the expression of p53 under the microscope the higher the ratio of mutated p53 in the specimen. See figures three and four on page 86.

For each patient three slides were made. Firstly a blank slide to ensure any positive results were due to the immunohistocytochemical staining for bcl-2 and p53. Secondly a slide was stained for bcl-2, and the third slide was stained for p53. Each slide was reviewed to determine whether it was positive for bcl-2 or p53. It is not possible to determine the sensitivity and specificity for the two reagents for bcl-2 or p53. The reason for this is that in order to determine the sensitivity and specificity of a test, the results must be measured against a known independent marker. As yet an independent marker of expression of bcl-2, and bax in tumour cells has not been found. There is not a quoted sensitivity or specificity in any of the published articles on this subject, and this is certainly an area where there is a gap in the knowledge.

If the slide was positive the degree of expression was ascertained. If the expression was visible on low power (x10) the biopsy was said to be strongly positive. If visible at medium power (x20) then the biopsy was moderately positive, and if high power (x40) was needed the biopsy was said to be weakly positive. In order to maximise the accuracy, and reproducabiliy of the results, all were repeated after a certain time interval, and all results were verified by a second party. Additionally the results were verified by a histopathologist experienced in this field.

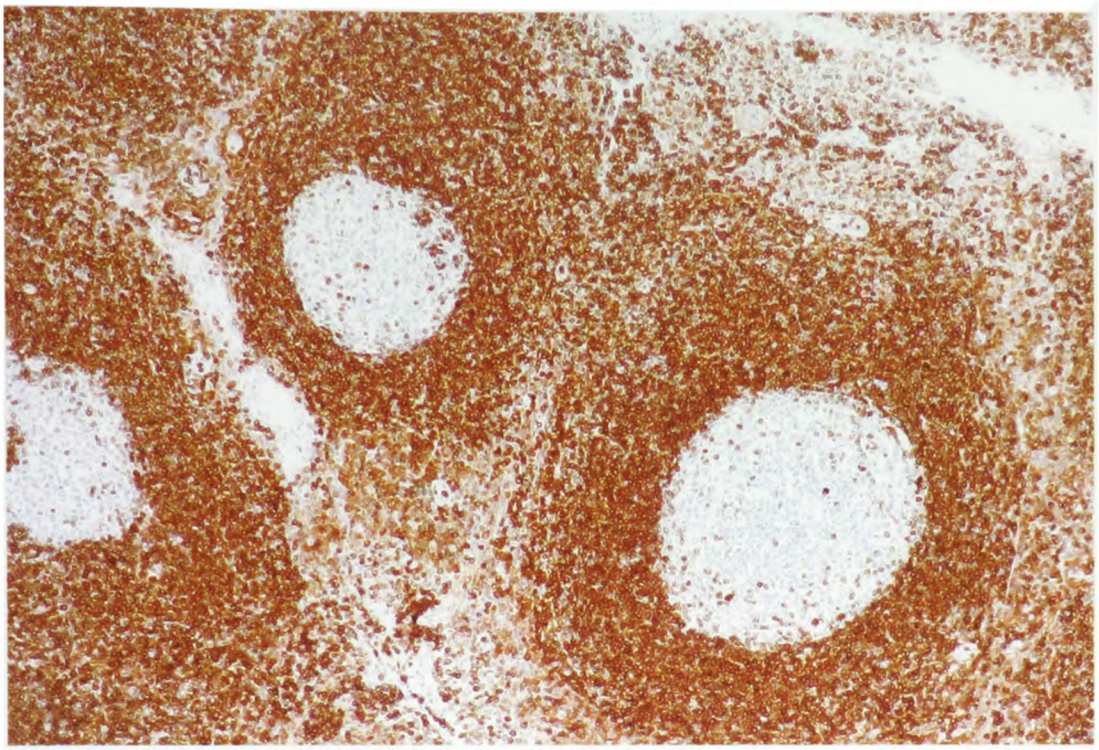

Figure Three. Tonsil control slide to show positive staining for bcl-2.

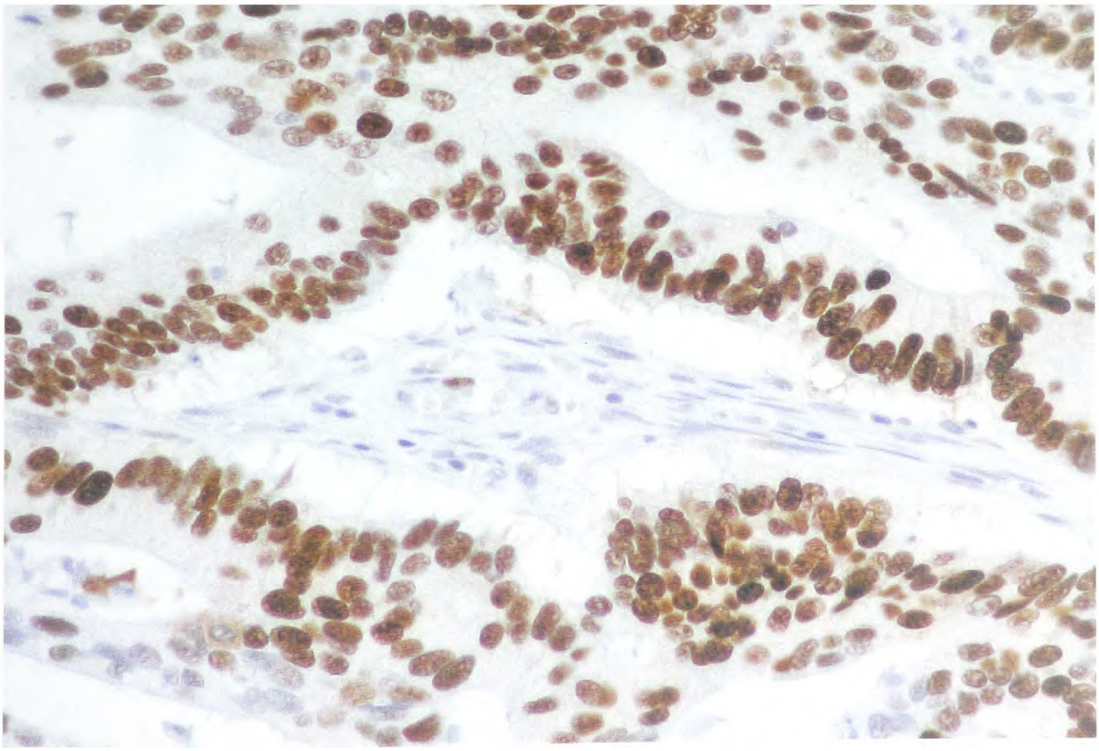

Figure Four. Colorectal cancer control slide to show positive staining for p53.

4.2.3 MORBIDITY AFTER NEOADJUVANT THERAPY

A retrospective review of the twelve patients' notes was carried out in order to determine whether those patients with a good response to the neoadjuvant treatment also had increased morbidity and mortality, as result of their tissues being sensitive to the treatment. The information collected was :-

1. Time the patient was ventilated on one lung during surgery.
2. Blood loss during theatre.
3. Length of stay in intensive care or high dependency unit post operatively and total inpatient stay.
4. Number of days the patient received antibiotics post operatively.
5. Inpatient morbidity/mortality.

4.3 RESULTS

4.3.1 bcl-2, p53 AND RESPONSE TO CHEMORADIOTHERAPY

The results are as shown in the following tables on page 88. There are two tables. The first shows the results for the patients having had a good response to chemoradiotherapy. The second table outlines the results for the patients who did not respond to chemoradiotherapy. In the column headed chemoradiotherapy (CRT) response is the histological response to the chemoradiotherapy in the resected cancer specimen. This was judged by the pathologist reporting the surgically resected specimen. Complete response showed absence of residual cancer. Near complete response was characterised by the presence of rare residual cancer cells, and significant residual disease was characterised by residual cancer outgrowing fibrosis. This is as described by Mandard *et al* in a previously discussed paper [51].

Histology	CRT response	bcl-2 expression	p53 expression
SCC	Near complete	Moderate	Strong
Adeno	Near complete	None	Strong
Adeno	Near complete	None	Strong
Adeno	Near complete	None	Strong
Adeno	Complete	None	Strong

Table Three. Expression of bcl-2 and p53 in those patients having a good response to chemoradiotherapy.

Histology	CRT response	Bcl-2 expression	p53 expression
SCC	Significant residual	None	None
Adeno	Significant residual	None	Strong
Adeno	Significant residual	None	Moderate
Adeno	Significant residual	None	None
Adeno	Significant residual	None	Strong
Adeno	Significant residual	None	Strong
Adeno	Significant residual	None	Strong

Table Four. Expression of bcl-2 and p53 in those patients having a poor response to chemoradiotherapy.

The figures on page 89 show two of the slides. Figure six shows an example of p53 positivity. The positive p53 staining can clearly be seen in the epithelial cells. Figure seven shows an example of a slide that is bcl-2 negative, although there is some functional expression demonstrated within the inflammatory cells. However there is no expression of bcl-2 within the epithelial cells.

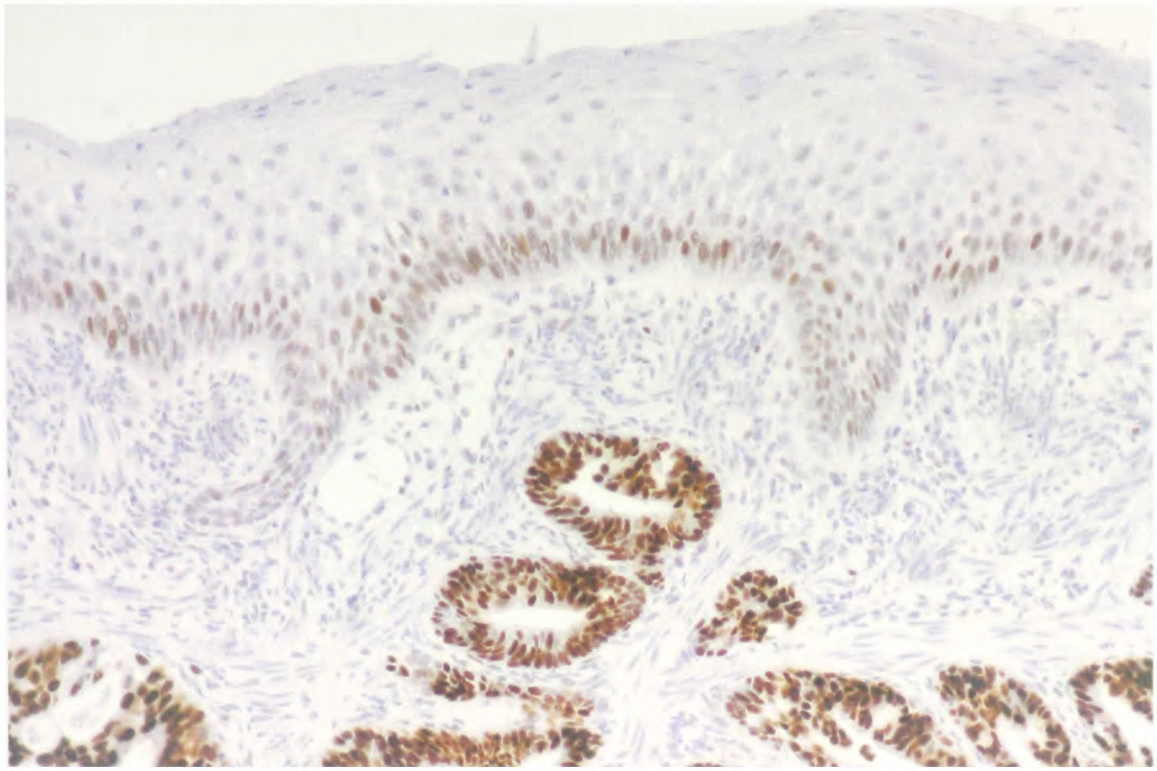

Figure Five. Slide of oesophageal cancer to show p53 positivity.

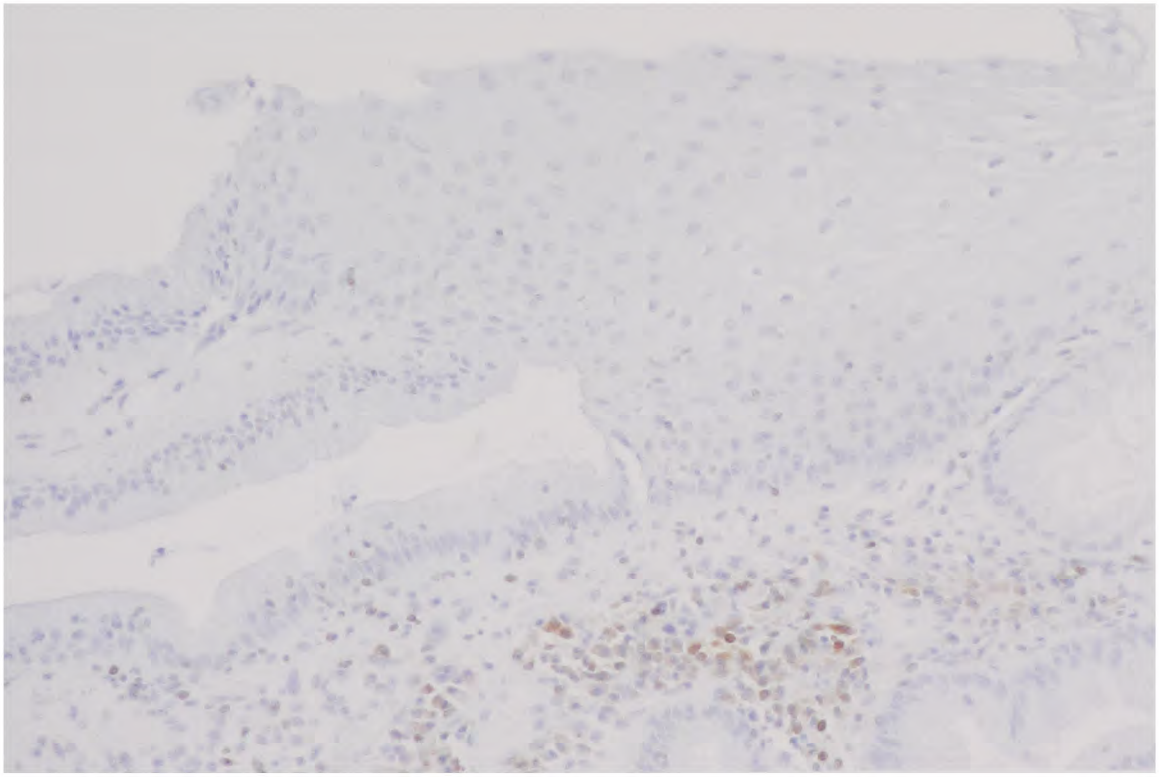

Figure Six. Slide of oesophageal cancer to show bcl-2 negavivity. Functional expression in inflammatory cells only.

4.3.2 MORBIDITY AFTER CHEMORADIOTHERAPY

Twelve patients had neoadjuvant chemoradiation before oesophagectomy. Four cycles of cisplatin were given every three weeks plus continuous infusion of 5-fluorouracil. Cycles three and four were given concurrently with 45 Gy external beam radiotherapy to the mediastinum delivered in 25 fractions over five weeks [44]. Radiotherapy to the mediastinum has been shown to cause cardiac damage [47], and pnemonitis [45, 46], which can result in increased post-operative complications [46].

Ten patients had adenocarcinoma and two patients had squamous cell carcinoma. Of these twelve patients five had a good response to the treatment (no residual tumour histologically in the specimen in one case and rare residual cancer cells in four cases) and seven had a poor response (tumour cells still present histologically). See Tables five and six on page 91. There was no difference between the ages and the histological type of tumour between the groups. There were no inpatient deaths in the group having a poor response to chemoradiation. There was one inpatient death in the group who had a good response. This death was due to adult respiratory distress syndrome, sepsis and pulmonary odema.

60% of patients had a complication in the group having a good response, compared to 42% having a poor response. The patients in the group responding well to chemoradiation had a far longer stay on both ITU and HDU and total hospital stay 21.5 days and 26.5 days, as compared to 9.5 days and 24.5 days for the group having a poor response. The time the patient was ventilated on one lung was the same for both groups, the group responding poorly had greater blood loss of 1100mls compared to 550 mls. The good responders had a far longer duration of antibiotic therapy. This was 22 days as compared to 7.5 days. The decision to continue antibiotics was made by the Consultant Surgeon on the basis of clinical judgement.

Age	Histology	Inpatient Mortality	Inpatient Morbidity	In hospital stay	ITU/ HDU	One lung time	Blood loss in theatre	Antibiotic days
48	SCC	N	Chylothorax Pleural effusion	42/7	8/7	90 mins	500mls	42/7
58	Adeno	N	N	14/7	6/7	90 mins	800mls	5/7
56	Adeno	Y	ARDS Sepsis Pulmonary odema	37/7	37/7	180 mins	400mls	37/7
34	Adeno	Y	Chyle leak thoracotomy	29/7	10/7	120 mins	750mls	7/7
38	Adeno	N	N	11/7	9/7	90 mins	300mls	2/7
Median		20%	60%	26.5/7	21.5/7	135 mins	550 mls	22/7

Table Five. Patients having good histological response to chemoradiation.

Age	Histology	Inpatient Mortality	Inpatient Morbidity	In hospital stay	ITU/ HDU	One lung time	Blood loss in theatre	Antibiotic days
54	Adeno	N	Small anastomotic leak	28/7	14/7	150 mins	500mls	11/7
53	Adeno	N	N	14/7	7/7	150 mins	400mls	8/7
50	Adeno	N	N	14/7	7/7	120 mins	400mls	5/7
51	Adeno	N	Empyema	35/7	6/7	120 mins	1800mls	7/7
56	Adeno	N	Pleural effusion	17/7	5/7	150 mins	1500mls	4/7
48	SCC	N	N	16/7	7/7	105 mins	400mls	5/7
45	Adeno	N	N	14/7	7/7	90 mins	500mls	5/7
Median		0%	42%	24.5/7	9.5/7	120 mins	1100mls	7.5/7

Table Six. Patients having poor response to chemoradiation.

4.4 DISCUSSION

Over expression of bcl-2 has been found in 72 % of Barrett's metaplastic lesions and in 100% of Barrett's with low grade dysplasia [72], and an elevated ratio of bcl-2 to bax in prostate cancer has been shown to predict an increased risk of the cancer failing to respond to radiotherapy [82]. Over expression of p53 has been shown to decrease the response to chemoradiotherapy [75], and forced expression of wild-type p53 gene can increase the sensitivity to chemoradiotherapy [77]. It has been shown that patients receiving neoadjuvant chemoradiotherapy may be at greater risk of infections [62], and have a reduction in physical performance which in turn can predict the subsequent postoperative risk [63].

This study was therefore set up to determine whether it would be possible in the clinical setting to determine the levels of bcl-2, bax and p53 in oesophageal tumours and use this information to predict whether the patient would be likely to respond to chemoradiotherapy. It was expected patients responding would have high levels of bax, and a bcl-2 to bax ratio favouring bax expression. Those not responding would have high levels of bcl-2, and p53 (as immunohistocytochemistry for p53 identifies mutated p53 only, and not wild-type p53). If it were possible to identify the patients not likely to respond they would be spared toxic treatment, and the possible increased post-operative complications associated with this.

It was discovered whilst beginning analysis that the antibody against the bax protein was not being expressed. All the oesophageal tumour biopsies being analysed had previously been fixed in paraffin, as part of the routine preparation of histological slides. Unfortunately it was realised the bax antigen, was undetectable in the paraffin sections. To rectify this a catalysed amplification system was necessary in order to enhance expression. This is the most sensitive of all the immunohistochemical

staining procedures, and involves the consecutive application of primary antibody, secondary link antibody, streptavidin-biotin complex, biotinylated tyramide, streptavidin peroxidase conjugate, and finally DAB substrate chromogen [178]. There were two reasons for the decision not to proceed with amplification. Firstly the ratio of bcl-2 to bax was more crucial than the individual expression of each. Amplification would mean the expression of bax being highly amplified as compared to the expression of bcl-2, rendering the ratio of one to the other impossible. Secondly purchasing the extra reagents would have imparted unforeseen substantial extra costs, with no guarantee of success. It was therefore decided to abandon the determination of bax expression, and hence the ratio of bcl-2 to bax. The study objectives were modified to ascertaining the expression of bcl-2 and p53 in the oesophageal tumour biopsies and comparing these to the response to chemotherapy.

Patients with oesophageal cancer treated with neoadjuvant chemoradiotherapy are divided into two distinct groups. There are those who respond well to treatment and have little or no residual tumour at operation, and there are those who do not respond and have residual disease. There was no obvious difference between the two groups in terms of expression of p53 or bcl-2. It is possible a difference exists, but very large numbers may be needed in order to demonstrate this. Of the papers identified on this subject several [76, 80, 81] identified no correlation between p53, and bcl-2 expression, and the response to chemotherapy.

The above results obtained suggest that patients having a good histological response to chemoradiation (because their tumours are sensitive to the treatment) may also have normal tissues which are sensitive, so resulting in an increase in mortality and morbidity. The patients in our series responding well to chemoradiotherapy had increased inpatient mortality, morbidity, ITU/HDU, and total

inpatient stay, and antibiotic days. The group responding poorly to chemoradiotherapy had an increased blood loss in theatre, this is most likely due to the residual tumour being more difficult to dissect. It has been shown in the literature that patients having neoadjuvant treatment may be at increased risk of infections and complications postoperatively [62, 63], in line with our findings.

4.5 CONCLUSIONS

1. Sadly because of unforseen methological problems, the results from the present study did not confirm the studies that have been highlighted in the review. Therefore as a result of this confounding variable no valid conclusion could be arrived at.

2. From the results of our small review it may be that patients having a good response to neoadjuvant chemotherapy have a higher rate of complications than those having a poor response.

CHAPTER FIVE

SUMMARY OF FINDINGS

DIRECTIONS FOR FUTURE RESEARCH

5.1 SUMMARY OF FINDINGS

1. The hypothesis was that patients with Barrett's oesophagus will have lower serum levels of antioxidants (Selenium, Copper, Zinc, Vitamins A, C, E, β-cryptoxanthine, and xanthophyll) than control groups. This hypothesis was found to be true with the serum levels of β- Cryptoxanthine, Vitamin C, selenium, and xanthophyll being significantly reduced in patients with Barrett's oesophagus as compared to control groups.

2. The hypothesis was that patients who have a good pathological and clinical response to neoadjuvant chemoradiotherapy will have a lower level of the apoptosis inhibiting oncogene bcl-2 in the tumour cells, and a bcl-2 to bax ratio favouring bax expression. The expression of bcl-2 and bax is a very exciting area, however the study because of unforeseen methological problems was not able to endorse the hypothesis.

3. The hypothesis was that the patients responding well to neoadjuvant therapy will have a high level of the bcl-2 inhibiting protein wild type p53. Unfortunately our results were unequivocal and we were unable to prove this hypothesis with our study.

5.2 DIRECTIONS FOR FUTURE RESEARCH

1. The next step on from this thesis would be a much larger-multi centred trial looking into the serum levels of antioxidants in patients with Barrett's oesophagus.

2. The trial described above could then be followed on with a supplementation trial whereby the diets of subjects with Barrett's oesophagus are supplemented with different antioxidants in particular selenium and vitamin C in order to ascertain whether supplementation could stop the progression to adenocarcinoma.

3. It would be very interesting to measure the antioxidant levels in patients having neoadjuvant chemoradiotherapy, in order to measure if there is a difference between those groups having a complete pathological response, and those having a poor pathological response to the treatment.

4. The use of bcl-2, bax, and p-53 being used as a responsive indicator to chemotherapy has major clinical implications. It would therefore be of extreme importance to repeat this experiment with a very tight control of the confounding variables.

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APPENDICES

Appendix I: Information sheet provided to patients recruited into study.

Project Title: A study to investigate the antioxidant profiles of patients with Barrett's oesophagus
(Version 3, 14th April 2003)

Patient Information Sheet

You are being invited to take part in a research study that is being undertaken by the Department of Surgery, Royal Glamorgan Hospital. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Consumers for Ethics in Research (CERES) publish a leaflet entitiled 'Medical Research and You'. This leaflet gives more information about medical research and looks at some questions you may want to ask. A copy may be obtained from CERES, PO Box 1365, London, N16 0BW. Thank you for reading this

Why have I been chosen?

Your consultant has recommended that you undergo an endoscopy examination of your stomach, to investigate the cause of your symptoms. An endoscopy is a flexible camera examination of the oesophagus (gullet) and stomach. This is either performed with local anaesthetic spray to the back of your throat or with an injection of a sedative to make you sleepy. The potential risks of having an endoscopy are extremely small, and include bleeding or perforation (making a tear in the oesophagus or stomach). The risk of either of these occurring is 0.02%, that is a 1 in 5000 chance.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. You will keep a copy of the signed consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

What will happen to me if I take part?

As part of this research project you would be required to answer a few simple questions about yourself and have 15 ml of blood (1 tablespoon) taken from a vein in your arm or the back of your hand.

What are the side effects of taking part?

The possible side effects of taking the blood are minor pain or bruising at the site where the needle is inserted.

What are the possible benefits of taking part?
The information we get from this study may help us understand acid reflux disease better and may help us to treat future patients with this condition.

What if something goes wrong?
If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action, but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of the study, the normal National Health Service complaints mechanisms may be available to you.

Will my taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential. Any information which leaves the hospital will have your name and address removed so that you cannot be recogmised. The permission of your consultant has been obtained so that you can be invited to participate in the research study.

What will happen to the results of the research study?
We hope that the results will be available in about 18 months time and that they will be published in a medical journal. If you would like a copy, please let us know.

Who is organising and funding the research?
The research is not sponsored by any company or research organisation.

Who has reviewed the study?
Bro-Taf Local Research Ethics Committee have reviewed the study.

Contact for further information
Whilst taking part in our research, if you have any problems, please contact Mr D.J. Bowrey or Mr T.J. Havard, Department of Surgery, Royal Glamorgan Hospital by telephoning 01443-443542.

Project Title: A study to investigate the antioxidant profiles of patients with Barrett's oesophagus
(Version 3, 14th April 2003)

I have read the Patient Information Sheets and agree to participate in the study.

The nature of the study has been fully explained to me by:_____

Signature of responsible physician:_____

Name of patient:_____

Signature of patient:_____

Signature witnessed by (name):_____

Signature of witness:_____ Date:_____

Appendix II: Raw Data for Antioxidant Patients.

	A	B	C	D	E	F	G	H	I
1	Study Num	Hospital Nu	Date of Bir	Sex	Age	Date of En	Type	Study Grou	Heartburn
2	1	M2535976	########	Male	57	########	Out patient	Normal Co	FALSE
3	2	M0171685	########	Male	55	########	Out patient	Reflux, no	FALSE
4	4	M2050877	########	Female	51	########	Out patient	Normal Co	TRUE
5	5	M2168963	########	Female	43	########	Out patient	Normal Co	TRUE
6	6	M2082544	########	Female	65	########	Out patient	Normal Co	TRUE
7	7	W0075012	########	Male	52	########	Open Acce	Reflux, no	TRUE
8	9	M2147551	########	Male	37	########	Out patient	Normal Co	TRUE
9	10	M0223884	########	Male	52	########	Open Acce	Normal Co	TRUE
10	11	M2030896	########	Female	49	########	Open Acce	Barrett's(s)	TRUE
11	12	M2534711	########	Male	63	########	Open Acce	Reflux, no	TRUE
12	13	M2525721	########	Female	53	########	Open Acce	Normal Co	TRUE
13	14	M2115011	########	Male	61	########	Open Acce	Reflux, no	TRUE
14	15	M2041461	########	Male	60	########	Open Acce	Reflux, no	TRUE
15	18	M2143349	########	Male	47	########	Open Acce	Normal Co	TRUE
16	19	M0151930	########	Male	24	########	Out patient	Normal Co	FALSE
17	20	M2161592	########	Male	73	########	Out patient	Barrett's(s)	FALSE
18	22	M2018672	########	Male	50	########	Open Acce	Barrett's(s)	TRUE
19	23	M2165410	########	Female	36	########	Out patient	Normal Co	TRUE
20	25	M2043796	########	Male	59	########	Out patient	Barrett's(s)	TRUE
21	28	M2502732	########	Female	53	########	Out patient	Reflux, no	TRUE
22	29	M2528679	########	Female	85	########	In-patient	Barrett's(s)	FALSE
23	32	M2537216	########	Male	58	########	Open Acce	Reflux, no	TRUE
24	35	M2066188	########	Female	45	########	Open Acce	Normal Co	TRUE
25	36	M2094092	########	Female	73	########	Open Acce	Reflux, no	TRUE
26	37	M2543793	########	Female	72	########	Open Acce	Normal Co	FALSE
27	38	M2099442	########	Female	50	########	Open Acce	Reflux, no	TRUE
28	40	M2142340	########	Female	20	########	Open Acce	Normal Co	TRUE
29	41	M0077486	########	Female	70	########	In-patient	Normal Co	FALSE
30	42	M2009657	########	Female	40	########	Out patient	Normal Co	TRUE
31	45	M2133233	########	Male	60	########	Open Acce	Reflux, no	FALSE
32	47	M2134216	########	Male	41	########	Out patient	Barrett's(s)	FALSE
33	48	M2163460	########	Male	43	########	Open Acce	Barrett's(s)	FALSE
34	49	M2019265	########	Male	49	########	Open Acce	Barrett's(s)	FALSE
35	50	M2029375	########	Male	45	########	Out patient	Barrett's(s)	FALSE
36	51	M2169437	########	Male	38	########	Out patient	Normal Co	TRUE
37	52	M2074647	########	Female	25	########	Open Acce	Normal Co	TRUE
38	53	M0222896	########	Male	50	########	Out patient	Barrett's(s)	FALSE
39	55	M2169182	########	Male	60	########	Open Acce	Reflux, no	FALSE
40	56	M2013272	########	Female	57	########	Open Acce	Reflux, no	FALSE
41	58	M2500563	########	Female	67	########	Open Acce	Normal Co	FALSE
42	59	M2032862	########	Female	60	########	Open Acce	Reflux, no	FALSE
43	60	M2169275	########	Male	44	########	Open Acce	Reflux, no	TRUE
44	61	M2518147	########	Male	77	########	Open Acce	Reflux, no	TRUE
45	63	M2013564	########	Female	51	########	Open Acce	Normal Co	TRUE
46	64	M2541006	########	Female	42	########	Open Acce	Normal Co	TRUE
47	67	M2146756	########	Male	58	########	Out patient	Barrett's(s)	FALSE
48	68	M2027272	########	Male	55	########	Out patient	Barrett's(s)	FALSE
49	69	M0032981	########	Male	42	########	Out patient	Barrett's(s)	FALSE
50	71	M2027610	########	Female	55	########	Open Acce	Reflux, no	TRUE
51	73	M1039028	########	Female	47	########	Open Acce	Normal Co	FALSE
52	74	M2079610	########	Male	51	########	Out patient	Barrett's(s)	TRUE

Appendix II: Raw Data for Antioxidant Patients.

	A	B	C	D	E	F	G	H	I
53	75	M2002802	########	Female	57	########	Open Acce	Reflux, no	TRUE
54	76	M1043879	########	Male	53	########	Out patient	Normal Co	FALSE
55	77	M2015280	########	Female	63	########	Open Acce	Reflux, no	FALSE
56	78	M2513681	########	Male	40	########	Open Acce	Reflux, no	TRUE
57	80	M2145962	########	Male	61	########	Open Acce	Barrett's(s)	TRUE
58	81	M2518063	########	Male	52	########	Out patient	Barrett's(s)	FALSE
59	83	M2032607	########	Male	64	########	Out patient	Reflux, no	TRUE
60	85	M2082946	########	Male	38	########	Open Acce	Normal Co	FALSE
61	87	M2169435	########	Female	61	########	Open Acce	Reflux, no	TRUE
62	90	M2169512	########	Male	55	########	Open Acce	Reflux, no	TRUE
63	91	M2523320	########	Female	54	########	Open Acce	Normal Co	TRUE
64	93	M2158304	########	Male	48	########	Out patient	Barrett's(s)	FALSE
65	94	M2515984	########	Male	48	########	Out patient	Barrett's(s)	FALSE
66	95	M2076754	########	Male	47	########	Out patient	Barrett's(s)	TRUE
67	96	M2538280	########	Male	39	########	Out patient	Barrett's(s)	TRUE
68	97	M2021058	########	Male	75	########	Open Acce	Barrett's(s)	TRUE
69	100	M2512423	########	Male	68	########	Open Acce	Normal Co	TRUE
70	101	W0001071	########	Male	41	########	Open Acce	Normal Co	TRUE
71	102	M2016673	########	Male	57	########	Open Acce	Reflux, no	FALSE
72	107	M2096984	########	Male	48	########	Open Acce	Barrett's(s)	FALSE
73	109	M2078053	########	Male	69	########	Open Acce	Reflux, no	FALSE
74	110	M2168606	########	Female	60	########	Open Acce	Normal Co	TRUE
75	111	M2041085	########	Female	64	########	Open Acce	Normal Co	TRUE
76	112	M2133707	########	Male	58	########	Open Acce	Normal Co	TRUE
77	113	M2137840	########	Male	53	########	Open Acce	Reflux, no	FALSE
78	114	M2023321	########	Male	58	########	Open Acce	Barrett's(s)	TRUE
79	115	M2545380	########	Male	55	########	Open Acce	Normal Co	TRUE
80	117	M2160712	########	Male	78	########	Open Acce	Barrett's(s)	FALSE
81	118	M2048038	########	Female	61	########	Open Acce	Reflux, no	TRUE
82	119	M3001971	########	Female	48	########	Open Acce	Normal Co	TRUE
83	120	M0098303	########	Female	54	########	Open Acce	Normal Co	TRUE
84	121	M3052553	########	Male	51	########	Open Acce	Barrett's(s)	TRUE
85	122	M2001211	########	Male	63	########	Out patient	Barrett's(s)	TRUE
86	123	M2135490	########	Male	78	########	Out patient	Barrett's(s)	FALSE
87	124	M2054891	########	Male	55	########	Out patient	Barrett's(s)	FALSE
88	125	M2551083	########	Female	60	########	Open Acce	Reflux, no	TRUE
89	126	M2075084	########	Male	73	########	Open Acce	Reflux, no	FALSE
90	127	M2164061	########	Female	56	########	Open Acce	Reflux, no	TRUE
91	128	M2112430	########	Male	35	########	Out patient	Reflux, no	TRUE
92	129	M2015406	########	Female	54	########	Out patient	Barrett's(s)	FALSE
93	130	M2015028	########	Male	67	########	Open Acce	Barrett's(s)	FALSE
94	131	M2069018	########	Male	66	########	Out patient	Barrett's(s)	FALSE
95	133	M2519820	########	Male	57	########	Out patient	Barrett's(s)	FALSE
96	134	M2107645	########	Male	61	########	Out patient	Barrett's(s)	FALSE
97	135	M2510453	########	Male	73	########	Out patient	Reflux, no	TRUE
98	136	M2019172	########	Male	43	########	Open Acce	Normal Co	TRUE
99	138	M2164689	########	Male	47	########	Open Acce	Normal Co	TRUE
100	139	M2143698	########	Male	61	########	Open Acce	Reflux, no	FALSE
101	140	M2013981	########	Male	44	########	Open Acce	Normal Co	TRUE
102	141	M2051352	########	Male	69	########	Out patient	Barrett's(s)	FALSE
103	142	M2011800	########	Male	74	########	Out patient	Barrett's(s)	FALSE
104	143	M2027663	########	Male	44	########	Open Acce	Barrett's(s)	TRUE

Appendix II: Raw Data for Antioxidant Patients.

	J	K	L	M	N	O	P	Q	R
1	HfreqD	HfreqW	HfreqM	HdurW	HdurM	Regurgitati	RfreqD	RfreqW	RfreqM
2	0	2	0	99	24	FALSE	0	0	0
3	2	0	0	0	99	FALSE	0	0	0
4	2	0	0	12	3	TRUE	0	0	2
5	6	0	0	12	3	FALSE	0	0	0
6	1	0	0	12	3	TRUE	0	1	0
7	0	3	0	99	24	FALSE	0	0	0
8	0	0	1	6	1.5	FALSE	0	0	0
9	1	0	0	99	24	TRUE	0	1	0
10	1	0	0	0	36	TRUE	1	0	0
11	3	0	0	0	36	TRUE	0	0	1
12	0	5	0	36	7	FALSE	0	0	0
13	3	0	0	99	24	FALSE	0	0	0
14	5	0	0	75	18	TRUE	0	3	0
15	1	0	0	50	12	TRUE	0	1	0
16	0	0	0	0		TRUE	1	0	0
17	0	0	0	0		FALSE	0	0	0
18	0	1	0	99	24	FALSE	0	0	0
19	0	0	1	24	6	TRUE	1	0	1
20	2	0	0	99	24	FALSE	0	0	0
21	1	0	0	0	60	TRUE	1	0	0
22	0	0	0	0		FALSE	0	0	0
23	0	2	0	52	12	TRUE	0	2	0
24	3	0	0	52	12	TRUE	0	0	3
25	3	0	0	99	24	TRUE	3	0	0
26	0	0	0	0		TRUE	0	0	1
27	3	0	0	52	12	TRUE	0	3	0
28	3	0	0	99	24	TRUE	3	0	0
29	0	0	0	0		TRUE	1	0	0
30	1	0	0	0	36	FALSE	0	0	0
31	0	0	0	0		FALSE	0	0	0
32	0	0	0	0		FALSE	0	0	0
33	0	0	0	0		FALSE	0	0	0
34	0	0	0	0		FALSE	0	0	0
35	0	0	0	0		FALSE	0	0	0
36	3	0	0	0	36	TRUE	3	0	0
37	1	0	0	25	6	TRUE	0	3	0
38	0	0	0	0		FALSE	0	0	0
39	0	0	0	0		FALSE	0	0	0
40	0	0	0	0		FALSE	0	0	0
41	0	0	0	0		FALSE	0	0	0
42	0	0	0	0		FALSE	0	0	0
43	1	0	0	99	24	TRUE	0	0	1
44	3	0	0	32	7	FALSE	0	0	0
45	0	1	0	16	4	FALSE	0	0	0
46	3	0	0	99	24	TRUE	0	1	0
47	0	0	0	0		FALSE	0	0	0
48	0	0	0	0		FALSE	0	0	0
49	0	0	0	0		FALSE	0	0	0
50	2	0	0	12	3	FALSE	0	0	0
51	0	0	0	0		TRUE	0	0	2
52	3	0	0	99	24	TRUE	0	1	0

Appendix II: Raw Data for Antioxidant Patients.

	J	K	L	M	N	O	P	Q	R
53	2	0	0	50	12	TRUE	1	0	0
54	0	0	0	0		TRUE	0	1	0
55	0	0	0	0		FALSE	0	0	0
56	3	0	0	99	24	TRUE	1	0	0
57	0	1	0	0	72	TRUE	0	1	0
58	0	0	0	0		FALSE	0	0	0
59	1	0	0	99	24	FALSE	0	0	0
60	0	0	0	0		TRUE	0	0	1
61	1	0	0	24	6	TRUE	0	2	0
62	0	4	0	52	12	FALSE	0	0	0
63	1	0	0	52		FALSE	0	0	0
64	0	0	0	0		FALSE	0	0	0
65	0	0	0	0		FALSE	0	0	0
66	3	0	0	99	24	FALSE	0	0	0
67	0	2	0	8	2	FALSE	0	0	0
68	3	0	0	26	6	TRUE	1	0	0
69	6	0	0	26	6	FALSE	0	0	0
70	1	0	0	99	24	TRUE	1	0	0
71	0	0	0	0		FALSE	0	0	0
72	0	0	0	0		FALSE	0	0	0
73	0	0	0	0		FALSE	0	0	0
74	0	0	1	16	4	FALSE	0	0	0
75	0	1	0	3	1	FALSE	0	0	0
76	0	0	1	52	12	FALSE	0	0	0
77	0	0	0	0		FALSE	0	0	0
78	3	0	0	4	1	FALSE	0	0	0
79	1	0	0	30	7	FALSE	0	0	0
80	0	0	0	0		FALSE	0	0	0
81	1	0	0	32	8	TRUE	1	0	0
82	0	1	0	16	4	TRUE	0	1	0
83	3	0	0	99	24	TRUE	0	1	0
84	1	0	0	0	60	FALSE	0	0	0
85	1	0	0	0	36	FALSE	0	0	0
86	0	0	0	0		FALSE	0	0	0
87	0	0	0	0		FALSE	0	0	0
88		1	0	52	12	FALSE	0	0	0
89	0	0	0	0		FALSE	0	0	0
90	1	0	0	99	24	FALSE	0	0	0
91	1	0	0	52	12	FALSE	0	0	0
92	0	0	0	0		TRUE	0	2	0
93	0	0	0	0		FALSE	0	0	0
94	0	0	0	0		FALSE	0	0	0
95	0	0	0	0		FALSE	0	0	0
96	0	0	0	0		FALSE	0	0	0
97	0	1	0	0	48	FALSE	0	0	0
98	1	0	0	28	7	FALSE	0	0	0
99	0	1	0	12	3	FALSE	0	0	0
100	0	0	0	0		TRUE	0	4	0
101	1	0	0	99	24	FALSE	0	0	0
102	0	0	0	0		FALSE	0	0	0
103	0	0	0	0		FALSE	0	0	0
104	1	0	0	0	150	FALSE	0	0	0

Appendix II: Raw Data for Antioxidant Patients.

	S	T	U	V	W	X	Y	Z	AA
1	RdurW	RdurM	Epigastric	EPfreqD	EPfreqW	EPfreqM	EPdurW	EPdurM	Dysphagia
2	0		TRUE	0	2	0	12	4	FALSE
3	0		TRUE	2	0	0	0	99	FALSE
4	12	3	FALSE	0	0	0	0		FALSE
5	0		FALSE	0	0	0	0		FALSE
6	12	3	TRUE	0	1	0	25	6	FALSE
7	0		FALSE	0	0	0	0		FALSE
8	0		FALSE	0	0	0	0		FALSE
9	100	24	FALSE	0	0	0	0		FALSE
10	150	36	FALSE	0	0	0	0		FALSE
11	50	12	TRUE	2	0	0	150	36	FALSE
12	0		TRUE	0	5	0	36	9	FALSE
13	0		FALSE	0	0	0	0		FALSE
14	75	18	TRUE	1	0	0	75	18	FALSE
15	50	12	TRUE	1	0	0	50	12	FALSE
16	150	18	FALSE	0	0	0	0		FALSE
17	0		FALSE	0	0	0	0		FALSE
18	0		FALSE	0	0	0	0		FALSE
19	50	11	TRUE	0	2	0	24	6	FALSE
20	0		FALSE	0	0	0	0		FALSE
21	52	12	TRUE	1	0	0	250	60	FALSE
22	0		FALSE	0	0	0	0		FALSE
23	52	12	FALSE	0	0	0	0		FALSE
24	16	4	FALSE	0	0	0	0		FALSE
25	150	36	FALSE	0	0	0	0		FALSE
26	0	3	TRUE	0	2	0	0	3	FALSE
27	8	2	FALSE	0	0	0	0		FALSE
28	100	24	FALSE	0	0	0	0		FALSE
29	150	36	FALSE	0	0	0	0		FALSE
30	0		FALSE	0	0	0	0		FALSE
31	0		FALSE	0	0	0	0		FALSE
32	0		FALSE	0	0	0	0		FALSE
33	0		FALSE	0	0	0	0		FALSE
34	0		FALSE	0	0	0	0		FALSE
35	0		FALSE	0	0	0	0		FALSE
36	150	36	TRUE	1	0	0	150	36	FALSE
37	250	60	FALSE	0	0	0	0		FALSE
38	0		FALSE	0	0	0	0		FALSE
39	0		TRUE	2	0	0	25	6	FALSE
40	0		FALSE	0	0	0	0		FALSE
41	0		FALSE	0	0	0	0		FALSE
42	0		FALSE	0	0	0	0		FALSE
43	99	24	FALSE	0	0	0	0		FALSE
44	0		FALSE	0	0	0	0		TRUE
45	0		FALSE	0	0	0	0		FALSE
46	99	24	FALSE	0	0	0	0		FALSE
47	0		FALSE	0	0	0	0		FALSE
48	0		FALSE	0	0	0	0		FALSE
49	0		FALSE	0	0	0	0		FALSE
50	0		FALSE	0	0	0	0		FALSE
51	25	6	FALSE	0	0	0	0		FALSE
52	99	24	FALSE	0	0	0	0		FALSE

Appendix II: Raw Data for Antioxidant Patients.

	S	T	U	V	W	X	Y	Z	AA
53	50	12	FALSE	0	0	0	0		FALSE
54	4	1	TRUE	0	0	3	4	1	FALSE
55	0		TRUE	2	0	0	25	6	TRUE
56	99	24	FALSE	0	0	0	0		FALSE
57	0	72	FALSE	0	0	0	0		FALSE
58	0		FALSE	0	0	0	0		FALSE
59	0		FALSE	0	0	0	0		FALSE
60	24	6	TRUE	0	1	0	24	6	FALSE
61	24	6	FALSE	0	0	0	0		FALSE
62	0		FALSE	0	0	0	0		FALSE
63	0		TRUE	0	2	0	26	6	FALSE
64	0		FALSE	0	0	0	0		TRUE
65	0		FALSE	0	0	0	0		FALSE
66	0		FALSE	0	0	0	0		FALSE
67	0		FALSE	0	0	0	0		FALSE
68	26	6	FALSE	0	0	0	0		FALSE
69	0		FALSE	0	0	0	0		FALSE
70	99	24	FALSE	0	0	0	0		FALSE
71	0		TRUE	1	0	0	8		FALSE
72	0		FALSE	0	0	0	0		FALSE
73	0		TRUE	2	0	0	8		FALSE
74	0		FALSE	0	0	0	0		FALSE
75	0		FALSE	0	0	0	0		FALSE
76	0		FALSE	0	0	0	0		FALSE
77	0		TRUE	1	0	0	12	4	FALSE
78	0		FALSE	0	0	0	0		FALSE
79	0		FALSE	0	0	0	0		FALSE
80	0		FALSE	0	0	0	0		FALSE
81	32	8	FALSE	0	0	0	0		FALSE
82	16	4	FALSE	0	0	0	0		FALSE
83	99	24	FALSE	0	0	0	0		FALSE
84	0		FALSE	0	0	0	0		FALSE
85	0		FALSE	0	0	0	0		FALSE
86	0		FALSE	0	0	0	0		TRUE
87	0		FALSE	0	0	0	0		FALSE
88	0		FALSE	0	0	0	0		FALSE
89	0		TRUE	0	1	0	8	2	TRUE
90	0		FALSE	0	0	0	0		FALSE
91	0		FALSE	0	0	0	0		FALSE
92	2	0.5	FALSE	0	0	0	0		FALSE
93	0		FALSE	0	0	0	0		FALSE
94	0		FALSE	0	0	0	0		FALSE
95	0		FALSE	0	0	0	0		FALSE
96	0		FALSE	0	0	0	0		FALSE
97	0		FALSE	0	0	0	0		FALSE
98	0		FALSE	0	0	0	0		FALSE
99	0		FALSE	0	0	0	0		FALSE
100	52	12	FALSE	0	0	0	0		TRUE
101	0		FALSE	0	0	0	0		FALSE
102	0		FALSE	0	0	0	0		FALSE
103	0		FALSE	0	0	0	0		FALSE
104	0		FALSE	0	0	0	0		FALSE

Appendix II: Raw Data for Antioxidant Patients.

	AB	AC	AD	AE	AF	AG	AH	AI	AJ
1	DfreqD	DfreqW	DfreqM	DdurW	DdurM	Chest Pain	CPfreqD	CPfreqW	CPfreqM
2	0	0	0	0		FALSE	0	0	0
3	0	0	0	0		FALSE	0	0	0
4	0	0	0	0		FALSE	0	0	0
5	0	0	0	0		FALSE	0	0	0
6	0	0	0	0		FALSE	0	0	0
7	0	0	0	0		FALSE	0	0	0
8	0	0	0	0		FALSE	0	0	0
9	0	0	0	0		FALSE	0	0	0
10	0	0	0	0		FALSE	0	0	0
11	0	0	0	0		FALSE	0	0	0
12	0	0	0	0		FALSE	0	0	0
13	0	0	0	0		FALSE	0	0	0
14	0	0	0	0		FALSE	0	0	0
15	0	0	0	0		FALSE	0	0	0
16	0	0	0	0		FALSE	0	0	0
17	0	0	0	0		FALSE	0	0	0
18	0	0	0	0		FALSE	0	0	0
19	0	0	0	0		FALSE	0	0	0
20	0	0	0	0		FALSE	0	0	0
21	0	0	0	0		FALSE	0	0	0
22	0	0	0	0		FALSE	0	0	0
23	0	0	0	0		FALSE	0	0	0
24	0	0	0	0		FALSE	0	0	0
25	0	0	0	0		FALSE	0	0	0
26	0	0	0	0		FALSE	0	0	0
27	0	0	0	0		FALSE	0	0	0
28	0	0	0	0		FALSE	0	0	0
29	0	0	0	0		FALSE	0	0	0
30	0	0	0	0		FALSE	0	0	0
31	0	0	0	0		FALSE	0	0	0
32	0	0	0	0		FALSE	0	0	0
33	0	0	0	0		FALSE	0	0	0
34	0	0	0	0		FALSE	0	0	0
35	0	0	0	0		FALSE	0	0	0
36	0	0	0	0		FALSE	0	0	0
37	0	0	0	0		FALSE	0	0	0
38	0	0	0	0		FALSE	0	0	0
39	0	0	0	0		FALSE	0	0	0
40	0	0	0	0		FALSE	0	0	0
41	0	0	0	0		FALSE	0	0	0
42	0	0	0	0		FALSE	0	0	0
43	0	0	0	0		FALSE	0	0	0
44	0	1	0	32	8	FALSE	0	0	0
45	0	0	0	0		FALSE	0	0	0
46	0	0	0	0		FALSE	0	0	0
47	0	0	0	0		FALSE	0	0	0
48	0	0	0	0		FALSE	0	0	0
49	0	0	0	0		FALSE	0	0	0
50	0	0	0	0		FALSE	0	0	0
51	0	0	0	0		FALSE	0	0	0
52	0	0	0	0		FALSE	0	0	0

Appendix II: Raw Data for Antioxidant Patients.

	AB	AC	AD	AE	AF	AG	AH	AI	AJ
53	0	0	0	0		FALSE	0	0	0
54	0	0	0	0		FALSE	0	0	0
55	3	0	0	25	5	FALSE	0	0	0
56	0	0	0	0		FALSE	0	0	0
57	0	0	0	0		FALSE	0	0	0
58	0	0	0	0		FALSE	0	0	0
59	0	0	0	0		TRUE	0	0	2
60	0	0	0	0		FALSE	0	0	0
61	0	0	0	0		FALSE	0	0	0
62	0	0	0	0		FALSE	0	0	0
63	0	0	0	0		FALSE	0	0	0
64	0	1	0	26	5	FALSE	0	0	0
65	0	0	0	0		FALSE	0	0	0
66	0	0	0	0		FALSE	0	0	0
67	0	0	0	0		FALSE	0	0	0
68	0	0	0	0		FALSE	0	0	0
69	0	0	0	0		FALSE	0	0	0
70	0	0	0	0		FALSE	0	0	0
71	0	0	0	0		FALSE	0	0	0
72	0	0	0	0		FALSE	0	0	0
73	0	0	0	0		FALSE	0	0	0
74	0	0	0	0		FALSE	0	0	0
75	0	0	0	0		FALSE	0	0	0
76	0	0	0	0		FALSE	0	0	0
77	0	0	0	0		FALSE	0	0	0
78	0	0	0	0		FALSE	0	0	0
79	0	0	0	0		FALSE	0	0	0
80	0	0	0	0		FALSE	0	0	0
81	0	0	0	0		FALSE	0	0	0
82	0	0	0	0		FALSE	0	0	0
83	0	0	0	0		FALSE	0	0	0
84	0	0	0	0		FALSE	0	0	0
85	0	0	0	0		FALSE	0	0	0
86	0	1	0	52	12	FALSE	0	0	0
87	0	0	0	0		FALSE	0	0	0
88	0	0	0	0		FALSE	0	0	0
89	0	1	0	8		FALSE	0	0	0
90	0	0	0	0		FALSE	0	0	0
91	0	0	0	0		FALSE	0	0	0
92	0	0	0	0		FALSE	0	0	0
93	0	0	0	0		FALSE	0	0	0
94	0	0	0	0		FALSE	0	0	0
95	0	0	0	0		FALSE	0	0	0
96	0	0	0	0		FALSE	0	0	0
97	0	0	0	0		FALSE	0	0	0
98	0	0	0	0		FALSE	0	0	0
99	0	0	0	0		FALSE	0	0	0
100	1	0	0	26	6	FALSE	0	0	0
101	0	0	0	0		FALSE	0	0	0
102	0	0	0	0		FALSE	0	0	0
103	0	0	0	0		FALSE	0	0	0
104	0	0	0	0		FALSE	0	0	0

Appendix II: Raw Data for Antioxidant Patients.

	AK	AL	AM	AN	AO	AP	AQ	AR	AS
1	CPdurW	CPdurM	Other Sym	OSfreqD	OSfreqW	OSfreqM	OSdurW	OSdurM	Endoscopy
2	0			0	0	0	0		Normal oes
3	0		0	0	0	0	0		Grade B oe
4	0		0	0	0	0	0		Normal
5	0		0	0	0	0	0		Normal
6	0		0	0	0	0	0		Normal
7	0		0	0	0	0	0		Oesophagi
8	0		belching	3	0	0	8	2	Normal,mil
9	0		0	0	0	0	0		3 duodena
10	0		0	0	0	0	0		Long segm
11	0		0	0	0	0	0		Oesophagi
12	0		0	0	0	0	0		Mild duode
13	0		0	0	0	0	0		Oesophagi
14	0		0	0	0	0	0		Oesophagi
15	0		0	0	0	0	0		Normal
16	0		0	0	0	0	0		Normal
17	0		0	0	0	0	0		Barrett's,4
18	0		0	0	0	0	0		10cm Barr
19	0		0	0	0	0	0		Normal
20	0		0	0	0	0	0		3cm Barre
21	0		0	0	0	0	0		Grade B oe
22	0		anaemia	0	0	0	0	0	Barrett's 1
23	0			0	0	0	0	0	Reflux oes
24	0			0	0	0	0	0	Normal oes
25	0			0	0	0	0	0	Grade B re
26	0			0	0	0	0	0	Normal
27	0			0	0	0	0	0	Grade A oe
28	0			0	0	0	0	0	Normal
29	0			0	0	0	0	0	Normal
30	0			0	0	0	0	0	Normal
31	0		redo on los	0	0	0	0	0	Grade C oe
32	0		on long ter	0	0	0	0	0	Barrett's 8c
33	0		on long ter	0	0	0	0	0	Barretts 8c
34	0		None, chec	0	0	0	0	0	Barretts 1c
35	0		none, on lo	0	0	0	0	0	Barrett's 1
36	0			0	0	0	0	0	Gastric ulc
37	0			0	0	0	0	0	Mild duode
38	0		Choking	0	0	0	0	0	Barrett's 6c
39	0			0	0	0	0	0	Grade A R
40	0		Nausea	1	0	0	25	6	Grade A re
41	0		On zoton	0	0	0	0	0	Mild duode
42	0		Vomiting	2	0	0	50	12	Reflux oes
43	0			0	0	0	0	0	Reflux Oes
44	0			0	0	0	0	0	Reflux Oes
45	0			0	0	0	0	0	Gastritis
46	0			0	0	0	0	0	HH, otherw
47	0		Controlled	0	0	0	0	0	Barretts 3c
48	0		Bloating	0	0	0	0	0	Barretts 1c
49	0		Controlled	0	0	0	0	0	Barretts 3c
50	0			0	0	0	0	0	Reflux Gra
51	0			0	0	0	0	0	Duodenitis
52	0			0	0	0	0	0	Barretts 8c

Appendix II: Raw Data for Antioxidant Patients.

	AK	AL	AM	AN	AO	AP	AQ	AR	AS
53	0			0	0	0	0	0	Reflux Gra
54	0			0	0	0	0	0	Normal
55	0			0	0	0	0	0	Grade B re
56	0			0	0	0	0	0	Grade B re
57	0			0	0	0	0	0	Barretts 2c
58	0		Controlled	0	0	0	0	0	Barretts 3c
59	0	120		0	0	0	0	0	Grade B re
60	0			0	0	0	0	0	Normal
61	0			0	0	0	0	0	Grade B re
62	0			0	0	0	0	0	Small HH a
63	0			0	0	0	0	0	Normal
64	0			0	0	0	0	0	Barrett's sh
65	0		Controlled	0	0	0	0	0	4cm Barret
66	0			0	0	0	0	0	HH and 1c
67	0			0	0	0	0	0	Short segm
68	0			0	0	0	0	0	Grade B R
69	0			0	0	0	0	0	Normal
70	0			0	0	0	0	0	Gastritis, d
71	0			0	0	0	0	0	Duodenitis
72	0			0	0	0	0	0	5cm Barret
73	0			0	0	0	0	0	Grade A re
74	0			0	0	0	0	0	Mild Gastri
75	0			0	0	0	0	0	Normal
76	0		Belching	3	0	0	52	12	Mild gastrit
77	0			0	0	0	0	0	Reflux Gra
78	0			0	0	0	0	0	4cm Barret
79	0			0	0	0	0	0	Multiple du
80	0			0	0	0	0	0	4cm barret
81	0			0	0	0	0	0	Reflux oes
82	0			0	0	0	0	0	Normal
83	0			0	0	0	0	0	Gastritis, H
84	0			0	0	0	0	0	Barrett's 1(
85	0			0	0	0	0	0	Barrett's4c
86	0			0	0	0	0	0	Barrett's
87	0		Controlled	0	0	0	0	0	Barretts
88	0			0	0	0	0	0	Grade A re
89	0			0	0	0	0	0	Reflux oes
90	0			0	0	0	0	0	Grade A re
91	0			0	0	0	0	0	Grade A re
92	0			0	0	0	0	0	Barrett's 2
93	0		Controlled	0	0	0	0	0	Barrett's 2(
94	0		Controlled	0	0	0	0	0	Barrett's 9(
95	0		Controooled	0	0	0	0	0	Barrett's 6
96	0		Controlled	0	0	0	0	0	Barrett's 6(
97	0			0	0	0	0	0	Grade A re
98	0			0	0	0	0	0	Normal en(
99	0			0	0	0	0	0	Gastritis
100	0			0	0	0	0	0	Grade D re
101	0			0	0	0	0	0	Gastritis
102	0		On Zoton	0	0	0	0	0	2cm Barret
103	0		On zoton	0	0	0	0	0	4cm Barret
104	0			0	0	0	0	0	Barrett's 2(

Appendix II: Raw Data for Antioxidant Patients.

	AT	AU	AV	AW	AX	AY	AZ
1	Hiatus herr	Size of her	Savaray-M	Intestinal m	Histology F	Albumin	CRP
2	FALSE			FALSE	Clo +ve	36	7
3	FALSE		B	FALSE		42	7
4	FALSE			FALSE		40	7
5	FALSE			FALSE		43	7
6	FALSE			FALSE		37	7
7	TRUE	Small <5cn	B	FALSE		44	7
8	TRUE	Small <5cm		FALSE	Clo -ve	39	7
9	FALSE			FALSE	Clo-ve	36	7
10	FALSE			TRUE		38	13
11	TRUE	Small <5cn	B	FALSE		41	7
12	FALSE			FALSE	Clo-ve	38	7
13	TRUE	Large	B	FALSE	Clo-ve	42	7
14	TRUE	Small <5cn	B	FALSE	Clo-ve	47	7
15	FALSE			FALSE	Clo-ve	38	7
16	FALSE			FALSE	Clo-ve	38	7
17	FALSE			TRUE		45	7
18	TRUE	Small <5cm		TRUE		40	7
19	FALSE			FALSE	Clo-ve	39	7
20	FALSE			TRUE		38	7
21	TRUE	Large	B	FALSE	Clo-ve	42	7
22	FALSE			TRUE		35	7
23	TRUE	Large	C	FALSE		36	7
24	TRUE	Large		FALSE	Clo-ve	40	7
25	TRUE	Large	B	FALSE		40	9
26	FALSE			FALSE		40	7
27	TRUE	Small <5cn	A	FALSE		43	10
28	FALSE			FALSE		42	7
29	TRUE	Large		FALSE		35	7
30	FALSE			FALSE	Clo-ve	35	7
31	TRUE	Large	C	FALSE		39	7
32	TRUE	Large		TRUE		38	7
33	TRUE	Small <5cm		TRUE		44	7
34	FALSE			TRUE		45	7
35	FALSE			TRUE		38	7
36	FALSE			FALSE		41	7
37	FALSE			FALSE	Clo-ve	36	16
38	FALSE			TRUE		44	9
39	FALSE		A	FALSE	Clo-ve	37	7
40	FALSE		A	FALSE		34	12
41	TRUE	Large		FALSE		40	7
42	TRUE	Small <5cn	B	FALSE		41	8
43	FALSE		B	FALSE	Clo-ve	40	7
44	FALSE		A	FALSE	Clo-ve	40	7
45	FALSE			FALSE	Clo-ve	40	7
46	TRUE	Large		FALSE	Clo-ve	43	7
47	TRUE	Small <5cm		TRUE		41	7
48	FALSE			TRUE		38	7
49	FALSE			TRUE		47	9
50	FALSE		A	FALSE		39	7
51	FALSE			FALSE	Clo-ve	36	7
52	TRUE	Large		TRUE		39	7

Appendix II: Raw Data for Antioxidant Patients.

	AT	AU	AV	AW	AX	AY	AZ
53	TRUE	Large	B	FALSE		39	10
54	TRUE	Small <5cm		FALSE		39	7
55	TRUE	Large	B	FALSE		36	9
56	FALSE		B	FALSE		44	7
57	FALSE			TRUE	Clo-ve	43	7
58	FALSE			TRUE		39	11
59	FALSE		B	FALSE		39	7
60	FALSE			FALSE		48	7
61	TRUE	Large	B	FALSE		40	13
62	TRUE	Small <5cn	B	FALSE		40	7
63	FALSE			FALSE		44	7
64	TRUE	Small <5cm		TRUE		38	8
65	FALSE			TRUE		42	7
66	TRUE	Small <5cm		TRUE	HP presen	38	9
67	FALSE			TRUE	Clo-ve	42	7
68	TRUE	Small <5cn	B	TRUE	metaplasia	39	7
69	FALSE			FALSE		37	7
70	FALSE			FALSE	Clo-ve	40	7
71	FALSE		B	FALSE	Clo +ve	43	7
72	FALSE			TRUE		38	11
73	FALSE		A	FALSE		39	14
74	FALSE			FALSE		43	7
75	FALSE			FALSE		36	7
76	FALSE			FALSE	Clo-ve	41	7
77	FALSE		B	FALSE		41	7
78	FALSE			TRUE		41	8
79	TRUE	Small <5cm		FALSE		43	8
80	TRUE	Small <5cm		TRUE		38	7
81	FALSE		A	FALSE		40	29
82	FALSE			FALSE		42	7
83	TRUE	Small <5cm		FALSE	Clo-ve	42	7
84	TRUE	Large		TRUE		38	10
85	FALSE			TRUE	Barretts, bu	41	7
86	FALSE			TRUE		39	12
87	FALSE			TRUE		39	7
88	FALSE		A	FALSE	Clo-ve	37	15
89	FALSE		B	FALSE		41	10
90	FALSE		A	FALSE		41	17
91	FALSE		A	FALSE		37	10
92	TRUE	Small <5cm		TRUE		35	7
93	TRUE	Small <5cm		TRUE		38	7
94	TRUE	Small <5cm		TRUE		37	7
95	TRUE	Large		TRUE		34	7
96	TRUE	Large		TRUE		39	7
97	TRUE	Small <5cn	A	FALSE		36	7
98	FALSE			FALSE		39	7
99	FALSE			FALSE		43	7
100	FALSE		D	FALSE		36	7
101	FALSE			FALSE	Clo-ve	41	7
102	TRUE	Large		TRUE		54	7
103	TRUE	Small <5cm		TRUE		42	12
104	TRUE	Small <5cn	B	TRUE		41	7

Appendix III: Publication and Presentation of Data.

DATA FROM CHAPTER THREE

Clements DM, Oleesky DA, Smith SC, Wheatley H, Hullin DA, Havard TJ, Bowrey DJ.
A study to determine plasma antioxidant concentrations in patients with Barrett's oesophagus.
J Clin Pathol 2005;58:490-492.

Clements DM, Oleesky DA, Smith SC, Wheatley HL, Hullin D, Havard TJ, Bowrey, DJ.
A study to determine serum antioxidant levels in patients with Barrett's oesophagus.
Presented by Mr. Bowrey at Society of Academic and Research Surgery.
Association of Upper GI Surgeons Symposium. Newcastle 2005.

Presented by myself at South Wales Gut Club April 2005.